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Food Technology Curricula

This is the fourth of a series of articles which are intended to elucidate the basic ideas that govern the curricula of our educational institutions in the field of food technology. The author or authors of each article belong to the staff of a Department of Food Technology which is actively training students. It is planned to include an article from each educational institution offering a curriculum in food technology. The article will describe the philosophy which guides the course of study and will describe the contents of the course. Included in the series later will be a report by the IFT Committee on Education and Curriculum, based upon the results of a questionnaire which was recently submitted to members of the IFT.

The Food Technology Curriculum at the University of Massachusetts

CARL R. FELLERS, ARTHUR S. LEVINE AND EDWARD E. ANDERSON

(Received for publication, May 19, 1950)

The Food Technology curriculum at the University of Massachusetts is designed to give the student a broad background in the related basic sciences as well as a more specialized concept in the study of foods. The student takes courses in chemistry, physics, microbiology and engineering throughout the four year undergraduate program. Courses in the Food Technology Department which deal specifically with food analysis, food development and manufacture are given in the junior and senior years. These advanced food technology courses include fundamental theories and practical applications that will provide the student with a technological approach to the problems that may be encountered in the food industries.

In addition to the four year undergraduate curriculum, advanced courses and research facilities are available for graduate work leading to the M.S. or Ph.D. degrees in Food Technology. An extensive graduate program is available to those students who qualify by virtue of a sound undergraduate record and who desire to continue their formal education.

By taking chemistry, bacteriology, nutrition or engineering, graduate students are able to broaden their field of study. Doctorate theses represent a careful, original investigation of fundamental food science problems. Theses to satisfy the requirements of a Master's Degree may be either fundamental or practical studies of problems dealing with foods or food processes.

A detailed curriculum for undergraduates seeking to major in food technology is outlined below:

Four-Year Undergraduate Curriculum for Food Technology Majors

FRESHMAN YEAR

1st Semester	*2nd Semester*
English Literature	continued
Speech	continued
Botany	Zoology
General Chemistry	continued
Mathematics	continued
Mechanical Drawing	Agronomy
Physical Education and Military	continued

SOPHOMORE YEAR

1st Semester	*2nd Semester*
English Literature	continued
Economics	Psychology
Introductory Bacteriology	continued
Qualitative Chemistry	Quantitative Chemistry
Physics	continued
Military	continued

JUNIOR YEAR

Introductory Food Technology	continued
Human Nutrition	Elements of Meat Packing
Organic Chemistry	continued
Determinative Bacteriology	Applied Food Bacteriology
Industrial Sanitation	Food Engineering
Chemical Engineering (Unit Processes)	Fisheries Technology

SENIOR YEAR

Industrial Food Technology	continued
Food Science Literature	continued
Examination of Food Products (analysis)	continued
Chemistry (Biological or Physical)	Confections and Cereal Products or Phy. Chem.
Marketing Practices	Introductory Research Methods or Dairy Products
Liberal Arts Elective	continued

In his junior year the student takes three department courses in food technology. *Introductory Food Technology*, a two semester course, outlines the subjects of food economics, production, distribution and processing and their relationship to the student's science background. Laboratory practice is obtained in canning, freezing and drying as well as in the handling of such specific products as pickles, maple products, citrus products, soups, condiments, meats and poultry. The properties and uses of sugar syrups, brines, enzymes, acids, salt, pectin, preservatives and antioxidants are also considered. *Fisheries Technology*, a single semester course, surveys the marine products of commerce with emphasis on New England's fisheries industry. Various methods of canning, curing, and freezing seafoods are studied with respect to their inherent chemical and microbiological problems.

As noted in the outline, juniors are urged to take courses related to food technology but offered by other departments of the University. These include *Industrial Sanitation, Food Engineering* and *Chemical Engineering* (Unit Processes), *Human Nutrition, Elements of Meat Packing,* and *Dairy Industry Courses.*

As seniors, students are required to take three full year courses, *Industrial Technology,* Seminar in *Food Science Literature* and *Examination of Food Products.* Further courses offered include *Confections* and *Cereal Products* and *Introductory Research Methods.*

The year's course in *Industrial Technology* surveys commercial practices in the manufacture and preservation of food products. Studies are made of factory arrangement, sanitation, government regulations and operation of commercial equipment in quantity production. The unit operations and processes involved in the production of frozen, canned, dehydrated and other manufactured food products are stressed.

Laboratory work deals with the manufacture and preservation of fish, meats, poultry, fruits and vegetables on a pilot plant level. Inspection and grading of processed foods is carried out in accordance with U. S. Standards and Food and Drug regulations. Several trips are made to food plants in the vicinity of Springfield as well as a field trip to Boston.

The courses in *Examination of Food Products* involve examining food products for adulteration, testing accessory products for quality, and determining the courses of spoilage or deterioration in processed foods. Physical, chemical, microbiological and microscopical methods are employed to this end. Other laboratory work includes the use of all kinds of laboratory, field equipment and apparatus used in food plants. Evaluation and interpretation of reported data are also stressed. Students successfully completing the above courses are well trained for specialized work in quality control, production and industrial research.

Food Science Literature involves acquainting students with the scientific and technical literature of the industry. Abstracts of current literature, special lectures, and round table discussions make up these seminar periods.

Introductory Research Methods is a course advocated especially for those students who intend to work for advanced degrees in Food Technology.

For graduate students specializing in the field of food technology advanced courses are offered. In addition to the basic undergraduate work these students take graduate courses as follows:

Thermal Processing of Foods
Freezing and Refrigeration
Food Packaging
Edible Fats and Oils
Unit Processes
Biological Assay of Foods
Graduate Seminar
Special Investigations
Thesis Research

Thermal Processing of Foods is an important subject for those who may go into the field of foods preserved by the application of heat. Heating characteristics of canned foods packed in tin containers and glass jars are studied. Processing times and temperatures are derived from studies of heat peneration data and thermal death points of spoilage microorganisms. Causes of spoilage and container failure are also considered.

Freezing and Refrigeration consists of a study of the various refrigeration systems, cold storage plant design, and methods used in quick freezing fruits, vegetables, meats and marine products.

Food Packaging includes a study of the characteristics of packaging materials with special emphasis on flexible films, paper, plastic and metal, and how they meet the packaging requirements of various food products. In the laboratory these materials are tested for structural quality, performance, moisture and gas transmission and other properties. Moisture equilibrium studies on food products are conducted as well as evaluation of commercially packaged items.

Edible Fats and Oils covers the production and uses of these products and their chemical nature in relation to their stability. The problem of oxidation and rancidity is considered with stress on causes of deterioration and methods of stabilization.

Unit Processes pertaining to the food industries are covered. The work includes instrumentation, temperature measurement, fluid flow, distillation, dehydration, filtration, comminution, deaeration and extraction.

Biological Assay of Foods provides laboratory training and practice in making animal and microbiological assays of such food constituents as vitamins and amino acids.

Current developments reported in sicentific journals are discussed in the *Graduate Seminar.* In *Special Investigations* graduate students have the opportunity to develop a research technic by studying a food problem prior to their more advanced investigations in *Thesis Research.*

Students trained at the undergraduate or graduate level with a curriculum as outlined above are thus equipped with a wide variety of knowledge related to food technology. Such students are qualified to assume positions of responsibility in food production and research in industry, government or educational institutions.

The success of the above program at the University of Massachusetts is confirmed by the record of over 300 graduates of the Department of Food Technology. In 1949 the department graduated 21 Bachelors of Science, 7 Masters of Science and 7 Doctors of Philosophy. In 1950 there were 18 Bachelors, 21 Masters and 10 Doctors in Food Technology. The Department of Food Technology is in the College of Agriculture of the University. The staff consists of two professors, two associate professors, three assistant professors, two instructors, two teaching fellows and eight research assistants.

Stephen Moulton Babcock

E. B. HART
University of Wisconsin, Madison, Wisconsin

(EDITOR'S NOTE: *As reported in the August issue of this journal, the 1950 Babcock award was presented to Carl R. Fellers during the Tenth Annual Convention of the Institute of Food Technologists in Chicago, Illinois. This biographical appreciation of Dr. Babcock, one of the outstanding American food technologists and for whom the Award was named, was prepared by Professor Hart for the Babcock Award Luncheon held on May 22, 1950. Professor Hart being unable to attend, the paper was read by Professor C. A. Elvehjem, also of the University of Wisconsin.)*

If Dr. Babcock were with us today he would wish that we have the merriest time possible. For as Glen Frank characterized him, "He was the laughing saint of Science."

Dr. Babcock spent more than forty years in our midst and it was a great experience to know him well. I first met him in 1906, the year in which I was asked to become associated with him in discharging the duties of the Department of Agricultural Chemistry. I shall never forget his trudging the streets with me looking for a house in which to live and taking as much personal interest in me as a father. I learned to know that that was his nature—a helpful spirit always giving sound counsel and always willing to lay aside his own work to discuss your problem. With my coming to the Department of Agricultural Chemistry he was freer to work on problems of no immediate practical moment, and we found him active in his laboratory on the fundamental question in plant and animal physiology, namely, metabolic water. This piece of work is now considered a classic by plant physiologists, and to the animal physiologist it explains how a clothes moth can live on dry clothes and produce larvae containing 75 percent of water. Dr. Babcock's grip on science was thorough, for he had superb training at Cornell University and the University of Göttingen, Germany, after having taken his A.B. degree at Tufts College. He brought to his problems a thorough understanding of mathematics, physics and chemistry.

He always delighted in telling his friends of his experiences in Göttingen University where he was a student of Huebner, the successor of the great chemist Wöhler. Wöhler was then a very old man, revered by the students who knew him, and almost daily walked through the laboratory where Dr. Babcock was at work. On one occasion Dr. Babcock asked a fellow German Student, "Who is that old duffer shuffling through here?" To be replied to in scorn, "Don't you know that that is the famous Wöhler?" In Wöhler Dr. Babcock found a most lovable man, and he never tired of telling how Wöhler showed him a sample of the first urea that was synthesized and a bit of iodine that was sent by Courtois in Paris to Wöhler. It was a sample of the first iodine that had been isolated and it was in a small sealed glass tube. The sample was so minute that it appeared as a small speck in the tube and only by warming the latter and causing the violent vapors to form could one be sure that the tube contained iodine.

Dr. Babcock also visited Bunsen at Heidelberg and was greatly impressed by the simplicity and originality of that great chemist. I have always suspected that much of the simplicity of his own experimental work grew out of the impressions he received from those early chemists.

In the popular mind Dr. Babcock will be remembered longest for his invention of the milk fat test which bears his name. He told me many times that his real contribution to the development of this test lay in his introducing the centrifuge as a part of the test, thereby shortening the time of operation. Here again his fundamental knowledge of physics stood him in good stead. Probably no contribution to agriculture by a scientific man has helped more to gain the respect and confidence of farmers in agricultural experiment stations than the Babcock test. But this inventive genius also developed a viscosimeter, the construction of which in principle is the basis of the modern viscosimeter. He chuckled and laughed as he told of this invention, for after it had been perfected to his satisfaction he found that the same principle and the same type of instrument had appeared in the German literature some twenty years before his own invention.

Dr. Babcock was a thoroughly religious man—if we may define a religious man as one who is humble before the Unknown, rather than a blind follower of theological ritual. In his early life he lost a job because he would not take part in the religious exercises prescribed for students at a certain eastern institution. He had been recommended to the position of chemist at this institution and while looking over the position was entertained at the president's house and was definitely offered the job. On the morning that he was to depart from the institution and was having breakfast with the president, the president said, "Now Mr. Babcock, there is one thing that I haven't mentioned to you and that is all members of our faculty take part in the daily chapel exercises held for our students, and of course you will be expected to take your part." Dr. Babcock, with a merry twinkle in his eye said, "Mr. President, if I come to this institution I come as a chemist and not to take part in these religious exercises." With that understanding he went back to Cornell University never expecting again to hear from the president of the eastern institution. In a few days he did hear that he had been appointed to the position, but he said that he was very glad that he could inform the president that he had already accepted a position at the Geneva Experiment Station.

Dr. Babcock was a thoroughly practical man although sometimes his advice bordered on the ridiculous. I well recall the incident of one of our faculty leaving for the Pacific Coast to lay out a new city below San Diego. Doctor was sought for advice on what he thought might make a model city. He believed that it would be impossible in that climate to have sufficient

milk for a growing population as produced under ordinary circumstances and so implored his friend to try the novel experiment of domesticating South Pacific whales to give up their milk to be pumped through a pipe line from the ocean slip. So far as I know the experiment was never carried out, but it illustrates the originality of the Doctor and his willingness to try what he often called "the fool experiment." He symbolized what A. V. Hill once said, "It is dangerous to speculate too far, but it is foolish not to speculate at all."

In Dr. Babcock's life there was no lull in activity and interest in scientific problems. He never seemed to grow old. At 87 he was still active on the problem of the constitution of matter, a problem which had always interested him since his student days at Cornell. It has always seemed to me that the many things he talked of in reference to the constitution of matter 30 and 40 years ago when I first knew him have come to be a part of modern physics and modern chemistry. True he was crude in this experimentation, but the ideas were there waiting for someone to prove or disprove. Physicists often quote the famous Morley experiment refined and carried out to the fifth decimal place as giving the atomic weight of oxygen. In some early experiments in weighing gases that Dr. Babcock himself carried out, he saw how a considerable error could creep in through the outside pressure upon the weighing flask, thereby distorting its shape. He wrote Morley asking if he had given consideration to this possible error in his own work, but Morley never replied, being conscious, I think, that he himself had actually committed a serious error. Dr. Babcock only chuckled and went on his way.

When he died he left a manuscript which we still have in the vault and which was the fruit of his labor and thought on the constitution of matter. I have read it through several times, and to me it was a new physics, but after submitting it to mathematicians and physicists upon our own campus with the thought of its publication, they were unanimous in believing that it was too qualitative in its character to warrant its publication and that possibly it might detract from Dr. Babcock's standing as a scientist.

After hearing their verdict I went to each one personally, not knowing enough about the subject myself, and said, "Isn't it possible that there is some hidden gem of thought in the manuscript that has escaped your critical attention and which might be lost if the manuscript were not published?" They all agreed that they did not think that to be the case. I still wonder whether they are right and whether our leading scientists may not often make the error of being grooved in certain channels of thought until they are roused from them by some wholly new or original idea.

His was the inquiring mind under all circumstances. When the tomatoes failed to grow in his garden the cause must be known. Instead of planting in a row, the next crop was planted in a circle and only those plants in the old row died. That row was underlain by an ant run and the cause of the failure solved.

Dr. Babcock received many honors. But I don't know of one from which he had more fun than what grew out of the Capper award. After it became known to the public that he was to receive $5,000 plus a medal, he was hounded by many people, including newspaper reporters as to what he expected to do with the money. In his very characteristic way he told them that he was going to buy peanuts with it, and of course this story got into the press. Out of it from all over the country came many appeals for financial help. It was evident that many believed that his mind had failed and that he could not possibly be responsible for the further handling of his own affairs. There wasn't a day that he didn't come into the laboratory with a letter or two to tell of the most recent request for help. One farmer in the north had suffered a great fire, his barns were all down, but he had enough money to rebuild everything but the hen coop—and if he could only have $500 he could rebuild the plant and again be on his way. A daughter was burdened with the care of an invalid mother; they were poor and needed help. The mother needed the out-door air, and if they could only have money enough for an automobile, health would be immediately restored. The Doctor would again laugh—and when he laughed, he laughed all over.

The present century has seen a great development in our knowledge of animal nutrition. Dr. Babcock's contribution to that development is not generally known. He lighted the torch for others to carry on. When chemist at the Geneva Experiment Station in 1882-1888 Dr. Sturtevant, the director of the station, wanted him to make some of the conventional analyses then and still in vogue on foodstuffs. The work involved not only the analysis of the food but also the analysis of some of the metabolic by-products. After he made the analyses and reduced the results to an ash free basis the composition of the food was much like that of the metabolic by-product. From that time on he lost faith in the possibility of the prevailing methods of food analysis to give valuable information about the nutritive value of a foodstuff. He also had little faith in the then prevailing and developing notion that the energy of a foodstuff would measure its nutritive value. The mind of the skeptic was again at work. He delighted in telling the American nutrition leaders of fifty years ago—Atwater, Armsby and Jordon, all champions of the idea that the energy of a food measured its nutritive value—that if energy were the measure, then hot water or coal should be the most excellent of foods. When he came to Wisconsin he put his ideas to work in testing with cows rations alike according to conventional methods of food analysis and energy content but selected from different sources, with marked differences in the resultant milk production and behavior of the animals. His notes were incomplete, but from no fault of his own, and so he never published the data. That work was really the forerunner of the larger development in America of the newer knowledge of nutrition, and the first experiment with large animals, so far as I know, using the biological method for testing the nutritive value of foodstuffs. It was a new idea and Dr. Babcock was father of the idea. Others have carried it on. Had the idea been lacking, we might still be in the hands of the energy exponents.

He had a profound influence upon the character of the scientific work done in our college, and possibly in the whole university. New ideas, free discussion, kindly interest, and above all his novel and helpful suggestions were an inspiration to many. He never threw a blanket over his apparatus when visitors came to see him. If interested, he told them fully what he was doing. When he suggested "the fool experiment" he started a new train of thought and out of it came a new birth. He would rather have an experiment fail than succeed if through its failure it taught him the plan for the next. Truth must always prevail.

Babcock was not a victim of the routine. The clock could not be regulated by his daily activities. Often he would come to the laboratory to tell me with much enthusiasm that he now had the solution of his problem—and then six months might elapse without reference to it. We thought he had forgotten about it, but not so. The incubation period was on; the first explanation was wrong; he was on a new track, and soon he would be bubbling over with new enthusiasm. His researches ran in cycles but never ceased.

He represented the liberal in research, and there can be little fundamental research if executive regulations dominate the time of men. His profound understanding and sympathetic nature made him a great teacher, and like Agassig, he is remembered by many whose questions were not answered but who were started on the way to learn for themselves through their own efforts.

Dr. Babcock published little. His influence was felt more through his interest in and suggestions to others. His counsel was always available and his ideas fresh and original. Fourscore years and more did not abate his interest in the experimental work of science. Babcock would have made any institution great, and it is Wisconsin's fortune to claim him for her fame.

Modesty and simplicity in practically everything were the characteristics of Dr. Babcock. He was a joyous comrade—a friend beloved beyond measure.

He pursued the most painstaking research as if he were playing a game. He brought laughter into the laboratory for there was about him that deceptively careless air which creative spirits have as they go about their work. He brought to his task that gaiety of spirit which authentic greatness can afford.

President Frank said of him:

"This grand old man of science was himself greater than anything he did, and thus he gives to us, the legatees of his spirit, a goal toward which to point the education and the science of our time.

"This merry man of many years was made of the stuff that gives mankind its saints and martyrs. But he was a saint without seriousness and he could have gone to martyrdom without a murmur of self pity, as part of the day's work. For his was a casual greatness."

Factors Influencing the Quality of Tomato Paste. II. Pectic Changes During Processing[a]

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(Received for publication, July 5, 1950)

Introduction

Consistency is one of the important factors influencing the quality and therefore marketability of tomato paste *(6, 8, 10)*. Attempts are frequently made to increase the consistency of thin pastes by increasing their solids content beyond the legally defined minimum of 25 percent. This practice cannot be very fruitful, as available data *(10)* indicate that solids play only a minor role in determining consistency.

It has been shown that pectic substances are of considerable importance in determining consistency in tomato juice *(6)* and, although tomato juice and tomato-paste processing methods differ, it seems plausible to assume that pectic substances may be of equal importance in their contribution to the consistency of tomato-pastes. In tomato juice mnaufacture, the freshly crushed tomato fruit (macerate) must be quickly heated to 180° F. (82° C.) *(6)* in order to inactivate pectic enzymes. If temperatures lower than this are employed, pectic enzymes remain active and rapidly alter the pectic substances, and the resulting tomato juice is characteristically thin and watery.

The pectic enzymes involved are pectin-methylesterase (PM) (also referrred to as pectinesterase) and pectic acid depolymerase (DP). The nature and action of these enzymes have been discussed elsewhere *(8)*; however, one important interrelationship of these enzymes will again be pointed out. The action of DP is primarily responsible for the type of breakdown affecting consistency in tomato products. DP is not easily inactivated by heat and is usually not completely destroyed at preheating temperatures. However, DP is essentially dependent on the action of PM to form its substrate, pectic acid, from the natural pectin of the tomato fruit. Thus inactivation of PM indirectly prevents the breakdown of pectic substances in tomato products, and the temperature of inactivation of PM,

[a] Presented before the Tenth Annual I. F. T. Convention, Chicago, Illinois, May 22, 1950.

[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

about 180° F. (82° C.), is therefore assumed to be the minimum heating temperature required for the prevention of losses in pectic substances.

The importance of inactivating the pectic enzyme system of the tomato fruit has not been fully appreciated in the tomato paste industry. This is in part due to an overemphasis of the importance of the solids contents and the fact that no adequate demonstration has been made either of the losses in pectic substances which may occur during paste processing or of the effect these losses have on the final consistency of the paste.

This paper reports the results of studies of changes undergone by the pectic substances of the tomato fruit in various stages of paste processing under the differing conditions encountered in nine plants in California, and shows the relationship of these changes to the consistency of the final pastes.

Experimental

In the operation of an average tomato-paste processing plant, the washed, trimmed tomato fruit is passed through a rotary disintegrator and the resulting macerate subsequently pumped into a "preheater" which may be either a tubular heat exchanger or a tank with a steam-heated mixing coil. The preheated macerate is then stored in one or more holding tanks which may or may not be also heated. Macerate from the holding tanks is "pulped" and "finished" and the resulting juice again stored in holding tanks which are usually of the heated type. These holding tanks supply the evaporators which concentrate the juice to at least 25 percent solids content as determined by refractive index measurements. The resulting tomato paste is then filled into cans, sterilized, and marketed.

Processing plants differ in the number of stages of holding and in the holding temperatures and orders of holding, pulping, and finishing. However, the most important plant differences, from the standpoint of pectic changes, are the temperatures attained in the preheated macerates and the rapidity with which preheating is accomplished. Preheater temperatures alone are of little significance, as the temperatures imparted to the macerates may vary considerably below or above this value depending on the volume and rate of flow.

Collection of Samples: Samples of crushed tomato macerate for pectin determinations and for testing for pectic enzyme were collected at a number of points in the processing lines of each plant visited. Processing lines in the tomato paste plants were often totally or partially enclosed, so that macerate collections were largely limited by the number and locations of accessible points in the line.

Samples for pectin analyses consisted of 50 ml. of tomato macerate, measured in a graduate, which were immediately added to 100 ml. of 95 percent ethanol in a screw-top bottle. Identical 25-ml. samples of macerate were taken at the same time for pectic enzyme tests. The latter samples were preserved at the time of collection by the addition of NaCl in excess of the amount necessary to saturate the liquid phase. Freshly canned paste samples were collected at the same time for pectin and other analyses and for consistency determinations.

Preparation of Samples: Since differences in temperature and occluded air in the macerate samples collected may have caused considerable error in the measurements of their volume, the samples for analyses were reduced to alcohol-insoluble solids (AIS) which could be compared on a basis of equal weights.

These samples, which were in about 65-70 percent alcohol, were brought to a boil to inactivate enzymes and after settling the supernatant liquid was decanted. The solids were washed in the container with 100 ml. of a 65-70 percent ethanol-water mixture containing 0.05 *N* HCl and filtered. The solids were washed three times on filter paper with portions of the 65-70 percent acid-ethanol mixture. The washed pulp was then dehydrated by washing with absolute ethanol, decolorized with ether, dried, ground, and weighed. The resulting AIS were stored in tightly closed bottles for pectin analyses.

Determination of Pectic Substances: Three types of pectic substances were extracted from the pastes and from the AIS prepared from the macerate samples, according to solubility properties *(7)*. The solvents employed were: (1) distilled water at 86° F. (30° C.); (2) 0.2 percent ammonium oxalate solution at 86° F. (30° C.); and (3) 0.05 *N* HCl at 185° F. (85° C.), in the order given. Two extractions with each solvent were employed. The material was mixed with a 50-ml. portion of the solvent in a 125-ml. Erlenmeyer flask for two hours at the specified temperature, and filtered. The filtrate was saved and the pulp washed back into the flask with another 50-ml. portion of solvent. After two hours, the extract was again filtered off and the pulp washed back into the Erlenmeyer flask with 50 ml. of the next solvent. The extractions were continued in this manner, yielding two extracts with each of the solvents under the conditions specified. The two filtrates (extracts) obtained with each solvent were combined and the pectic substances were precipitated by the addition of 2 volumes of 95 percent ethanol and sufficient HCl to make the mixture 0.05 *N*. The various pectic fractions were then determined separately as calcium pectate by the Carré-Haynes method *(4)*.

By this procedure, it was thought that differences relating to processing methods might be observed in the three types of pectic substances, namely, water-soluble pectin, oxalate-soluble pectic acid and low-ester pectins, and hot-acid-soluble protopectins occurring in the tomato fruit. The findings, however, do not justify detailing the analyses in this paper, so only the total pectic substances are reported.

It should be pointed out that calcium pectate determinations are subject to 5 to 10 percent errors due to impurities such as protein, which may occur in the calcium pectate precipitates.

Nevertheless, the method was chosen over other perhaps more accurate methods *(12, 13)* because only the high-molecular-weight pectic substances capable of contributing to consistency are included as calcium pectate. By other existing procedures, low-molecular weight pectic substances which do not contribute to consistency might also be included in the determinations.

Determination of the AIS: Accurately weighed 10-gram samples of tomato paste were added to 100 ml. of a 65-70 percent ethanol-water mixture made 0.05 *N* with HCl, vigorously stirred and allowed to stand overnight. The supernatant liquid was then filtered off and the process repeated two more times. After the last filtration, the pulp was washed on the filter paper with 95 percent ethanol, transferred to weighed drying pans, and dried overnight at 185° F. (85° C.) under a slow stream of dry air.

AIS were obtained indirectly in the macerate samples as the weights of the preparations made for pectin extractions; however, these weights are subject to sampling errors previously noted. In both cases, the ethanol level of 65-70 percent was arbitrarily chosen because of its relation to the precipitation of pectic substances *(5)*.

Rate of Filtration: The method employed was an adaptation of a method previously described for tomato juice *(6)*. Ten grams of paste were mixed with 50 ml. of distilled water and allowed to stand 30 minutes at 25° C. The mixture was then poured on sharkskin filter papers in 60° glass funnels of identical size and allowed to filter for 30 minutes. Rate of filtration is taken as the volume of filtrate obtained at the end of 30 minutes, and is inversely proportional to the thickness of the paste and the viscosity of the serum.

Rate of filtration is a quick, convenient, and moderately accurate method for estimating consistency: however, only the relative differences between samples run at the same time and under identical conditions should be considered as significant.

Insoluble Solids: The tentative A. O. A. C. method *(1)* for determining insoluble solids was employed except that 10-g. samples of paste were used and the soluble solids were extracted with 500-ml. portions of water as described. Filtrations were on sharkskin filter paper.

Total Solids: Total solids were determined as described in Bulletin 27-L Revised, of the National Canners Association *(3)*.

Pectic Enzymes: The macerate samples collected with NaCl added were filtered and PM and DP activities determined in 1-ml. portions.

PM activity was determined by the measurement of free carboxyl groups liberated as described by McColloch and Kertesz *(9)*. DP activities were determined by following the increase in reducing power at pH 4.5 of pectic acid-serum reaction mixtures employing the Willstätter-Schudel hypoiodite method as described by Bates and associates *(2)*.

Only PM activities are reported since, as stated earlier in the paper, inactivation of PM is sufficient to prevent (DP) activity *(8)*. DP was of course found to persist in the macerates beyond the point of PM inactivation.

Consistency: The consistency of the tomato-paste samples was determined by the Penetrometer[e] method described by Underwood and Keller *(11)*. Consistencies are given as the millimeters of penetration of a plastic cone into the paste, and the numerical value is inversely proportional to the consistency of the paste sample.

Results and Discussion

Effect of Preheating Temperature: In Table 1 are shown the results of the analyses of the tomato macerate samples collected during processing. The total pectic substances content of the pastes obtained the same day are also included in the table. Results are listed by plants in the order of decreasing consistency of the pastes obtained.

Because of the difficulties in collecting exactly comparable macerate samples, the proportion of pectic substances are expressed as percentages of the AIS of the macerates and of the pastes. However, AIS proved not to be an exactly fixed quantity in the macerates. This is in part due to the expected relatively small decreases caused by the loss of pectic substances which are a part of the AIS. However, even larger changes in AIS were found to occur in which there was a general decrease through succeeding stages of processing, being most marked where the preheating temperatures were below 170° F. (76.7° C.). As an extreme example, the AIS in the approximately 50-ml. macerate samples collected in Plant 9 varied from 1.8 percent at the crusher through 1.2 percent at the preheater discharge, 1.1 percent in the macerate entering the holding tank, and 0.8 percent in holding tank itself which had been filling for approximately two hours.

These changes are obviously greater than the errors which might be encountered in the collection of samples, and variations in the composition of the tomatoes employed. Preliminary investigations have indicated that these changes may be in part due to the alteration of proteins.

In Table 1 therefore the changes in pectic substances are shown on a compressed scale. Nevertheless, it is apparent that marked losses in pectic constituents occur when preheating temperatures are too low and PM is not inactivated (Plants 4 through 9). These losses are not always apparent in the macerate samples where they are collected in closely following phases of the manufacture (see Plant 4), nor are they necessarily related to the relative amount of PM activity present. In this connection, it should be pointed out that the actual destruction of pectic substances will depend not only upon the amount of enzyme present but also upon the temperature at which the enzymes are acting, on the time elapsed between taking the samples, and the inactivation of PM at later stages of processing (holding tank in plant 9). In all cases, however, the pectic substance contents of the final pastes reflect qualitatively the enzyme action present.

The apparent increases in the pectic substances in the pastes at Plants 5 and 6 are no doubt the results of errors in the method of analysis. Pastes are concentrated about 4-fold with respect to the macerate and the level of pectic substances found in the pastes would represent a few tenths of a milligram in the macerates. This small amount could scarcely be determined by the now available methods of analysis.

Since the analyses in Table 1 are presented in the order of decreasing consistency in the pastes, the effects of preheating temperatures are well illustrated.

An important conclusion apparent from the table is that the temperature of 180° F., recommended in tomato-juice manufacture, may not be adequate for the inactivation of pectic enzymes in the procedures employed in tomato-paste manufacture. This is in all probability the result of one of the major operational differences between juice and paste processing. In processing tomatoes for juice, the crushed macerate is usually "finished" almost immediately after crushing. Preheating is often accomplished during the crushing step or is performed on the "finished" juice. As a result, tomato juice is usually "finished" while at the preheating temperature or is actually preheated while in the finely divided "finished" form. The final juice therefore represents finely divided material which has been uniformly heated to the desired temperature and which has not been in contact with relatively large "chunks" of macerated pulp for any appreciable time.

By contrast, in the paste- manufacturing industry, it is the practice to preheat the unfinished macerate; later the mixture of juice and pulp may remain in contact for some time in one or more holding tanks before the "finishing" operation is performed. This practice may have the advantage of permitting the extraction of additional pectic substances into the liquid phase, but there is also the disadvantage of permitting the extraction of active pectic enzymes from large pieces of pulp which may not have been thoroughly heated. This effect appears most evident in the sample from Plant 4, which was employing the preheating temperature of 180° F. recommended for tomato juice. The PM was not completely inactivated in the preheater and there had been a distinct loss of pectic constituents by the time the macerate was processed into paste. Unfortunately, in this plant and in Plant 7, it was not possible to obtain samples of macerate between the preheater stage and the final paste to show the decreases in pectic contents which might be expected on the basis of the enzyme activity present. However, this type of loss is well illustrated in the data from Plants 8 and 9.

Effect of Pectic Changes on Consistency: The changes which may occur in the pectic substances of

[e] The mention of special instruments throughout this paper does not imply that they are endorsed or recommended by the Department of Agriculture over others of a similar nature not mentioned.

TABLE 1

Pectic Changes During Processing of Tomato Paste

Samples (by plants)	Identification of Samples	Tomatoes used	Temp. of macerate ° F.	PM units, relative	Pectic substances as calcium pectate in AIS
					%
	Fresh "Pear" Tomatoes		....	....	18.4
	Fresh "Round" Tomatoes		....	....	19.0
1	Crusher	Pears and rounds	....	21	12.0
	Tubular Preheater Discharge		210	0	11.1
	Final Paste		....	0	10.9
2	Crusher	Pears and rounds	....	19	9.6
	Tank Preheater Discharge		190	0	11.0
	Finisher Discharge		....	0	12.8
	Final Paste		....	0	11.7
3	Crusher	Rounds	....	11	11.5
	Tubular Preheater Discharge		Var. 175-185	2	9.9
	Holding Tank		210	0	9.1
	Final Paste		....	0	12.0
4	Crusher	Pears and rounds	....	39	16.1
	Tank Preheater Discharge		180	7	17.4
	Final Paste		....	0	3.2
5	Crusher	Rounds	....	38	8.2
	Tubular Preheater Discharge		150	24	0
	Holding Tank		185	5	0
	Final Paste		....	0	1.2
6	Crusher	Rounds	....	42	8.1
	Tubular Preheater Discharge		140	21	0
	Holding Tank		190	0	0.9
	Final Paste		....	0	1.2
7	Crusher	Pears and rounds	....	55	14.0
	Tubular Preheater Discharge		Var. 175-185	28	14.8
	Final Paste		....	0	1.4
8	Crusher	Rounds	....	31	11.1
	Entering Tank Preheater		....	31	12.4
	Tank Preheater Discharge		170	25	7.6
	Final Paste		....	0	0
9	Crusher	Rounds	....	21	13.4
	Tubular Preheater Discharge		Var. 165-170	15	2.6
	Holding Tank Inlet		140	15	1.8
	Holding Tank		140	11	0
	Final Paste		....	0	0

tomatoes during processing have been illustrated in Table 1. In order to demonstrate the effect of these changes on consistency, the paste samples are listed in Table 2, again in order of decreasing consistency, and with the PS content expressed as percentage on the wet basis. The rate of filtration and content of AIS, insoluble solids, and total solids are also given for comparison. The pectic substances and AIS contents of fresh Pearson (round) and fresh San Marzano (pear) tomatoes are also included. For these analyses, whole tomatoes were dropped into hot ethanol in a blender so that there was presumably no change in composition due to enzyme action. The AIS were weighed and pectic substances determined on the AIS as described earlier.

The relationship between pectic substances and consistency is apparent from Table 2. There is a general but not quantitative correspondence of lower consistencies with lower pectin contents in the pastes, and the lower pectin contents correspond in general to the decreasing preheating temperatures as shown in Table 1. An examination of the table indicates that rate of filtration appears to reflect the pectic substances contents of the pastes more closely. Thus rate of filtration might prove to be a simple, and effective, means for estimating consistencies in tomato pastes.

TABLE 2

Relationship of Pectic Substances and Alcohol-Insoluble Solids to Consistency In Tomato Paste

Sample	Pectic substances as calcium pectate (wet basis)	Consistency, by penetration	Rate of filtration [a]	AIS	Insol. Solids,	Total solids, (as dry matter)
	% x 10	*Mm.*		%	%	%
Fresh "pear" tomatoes	3.49	....	....	1.9	....	....
Fresh "round" tomatoes	2.47	....	....	1.3	....	....
Paste 1	4.46	261	7.5	4.1	2.9	24.0
Paste 2	5.25	263	7.5	4.5	3.5	22.8
Paste 3	3.72	265	11.0	3.1	2.8	23.3
Paste 4	1.12	273	21.3	3.5	3.4	24.7
Paste 5	0.36	284	29.5	3.0	2.7	24.2
Paste 6	0.37	285	27.5	3.1	2.6	23.1
Paste 7	0.43	285	24.0	3.1	3.0	24.3
Paste 8	0	305	30.0	2.8	2.6	21.9
Paste 9	0	313	30.0	2.4	2.4	22.8

[a] Filtrate in 30 minutes (ml.)

It is interesting to note in Table 2 that the AIS show a definite relationship to consistency and to the pectin contents of the pastes. Considering the concentration of paste (about 4 fold) and the AIS content of Pearson tomatoes, one might expect a minimum of 5 percent AIS in the paste samples. The values of AIS in the pastes range considerably below this and the differences are greater than the losses of pectic substances which are part of the AIS. Some of these differences may be accounted for by the removal of seeds and skin during finishing; however, as noted earlier in the paper, losses were found to be occurring even in the finished macerates. It is interesting to note that by the same token there is a loss of pectic substances even under the best operating conditions found.

The nature of the substances which may be undergoing change and the mechanism of the change are uncertain at present. Experimental results of Kertesz and McColloch *(7)* indicate that the AIS of fresh tomatoes contain about 10 percent pectic substances, 30 percent crude fiber, 5 percent ash, and 20-50 percent alcohol-insoluble nitrogen compounds calculated as proteins.

Aside from pectic substances, the proteins might be considered the most labile of the components found, and in combination with the fact that protein is the only apparent constituent of tomato AIS present in sufficient amount to account for the large changes observed, one might tentatively assume that proteins are also being broken down in some manner during the processing of tomato paste.

Since it is clearly evident from Table 2 that AIS play an important role in consistency, a study of the changes in AIS has been initiated in this laboratory.

Effect of Type of Tomato Employed: It is well known in the industry that the inclusion of "pear" tomatoes will considerably increase the consistency of tomato paste. The reason for this is apparent in Table 2 where the "pear" tomatoes are seen to contain larger amounts of both pectic substances and AIS, which have been shown to contribute significantly to consistency.

It should not be assumed that the use of "pear" tomatoes is always the best approach to increasing the consistency of tomato pastes. In Table 2, a comparison of the paste 3, which was processed from round tomatoes entirely, and paste 4, which was prepared from "pears" and "rounds" shows that even at the higher AIS content obtainable from pear tomatoes, the consistency may suffer if pectic substances are destroyed during processing. "Pear" tomatoes have less flavor than "rounds" and may impart a bitterness to tomato products in which they are included. In products where these factors preclude the use of pear tomatoes, the conservation of pectic substances will obviously be of even greater importance.

Effect of Other Components: The table well illustrates the minor role played by total solids in determining consistency. Thus in Table 2, one of the pastes of highest and one of lowest consistency (2 and 9 respectively) contain the same percentages of solids (22.8) and throughout the rest of the samples, solids contents vary more or less randomly with respect to consistency. Insoluble solids demonstrate a similar lack of relationship to consistency. It is possible that insoluble solids represent the non-colloidal high-molecular-weight compounds such as "crude fiber" which, lacking colloidal properties, do not contribute to consistency.

Summary

It has been shown that crushed tomato macerates retain active pectic enzymes if the macerates are not preheated to a sufficiently high temperature.

The preheating temperature required for pectic enzyme inactivation appears to be somewhate higher (185° F. or 85° C.) under the conditions of tomato paste manufacture than the 180° F. (82° C.) recommended in tomato juice production. The reasons for this difference are discussed.

Failure to inactivate pectic enzymes early in the processing procedure results in a rapid destruction of the pectic substances in the macerates, which in turn results in pastes of low pectin content and low consistency.

Evidence is obtained which indicates that some yet not well defined fractions at AIS are equally important contributors to consistency in tomato paste. AIS appear to undergo degredation during processing along with the degradation of PS, and pastes of low consistency contain lesser amounts of both pectic substances and AIS.

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Measurement of Nonenzymatic Browning of Dehydrated Vegetables During Storage

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Methods are presented for measuring degree of nonenzymatic browning in dehydrated white potato, carrot, cabbage, sweet potato, and onion. These methods are based on extraction of soluble colored materials from suitably ground samples, followed by clarification of the resultant extracts and photometric evaluation of the extracted color. Degree of browning is taken as the difference between optical density values for samples before and after storage.

Although the production of dehydrated vegetables was sharply curtailed at the end of the war, following its enormous wartime expansion, there exists today a sizeable vegetable dehydration industry. Uses have been developed and expanded for a number of dehydrated vegetables, including onions, carrots, and several different dried potato products, and hence dehydration appears assured of a permanent and perhaps increasingly important place as a method of vegetable preservation. Browning of the dehydrated products during storage has been a problem to the industry. Although this problem has received wide attention in recent years *(3, 5, 6, 8, 9, 11)*, for most dehydrated vegetables there have been no objective methods of measuring degree of browning. The development of such methods was required in this laboratory for evaluating the effects of storage temperature, moisture content, sulfite content, and similar factors on rate of browning, and for use also in measuring the protection against browning obtained when the moisture content is reduced by in-package desiccation *(4, 5, 7)*.

The methods developed, known as "soluble color" methods, were briefly described in a previous paper *(7)*. They consist of 3 steps: (1) extraction of soluble colored materials from suitably ground samples, (2) clarification of the resultant extracts, and (3) photometric evaluating of the extracted color. In the present paper factors of importance for these steps are discussed, and details of the procedures for white potato, carrot, cabbage, sweet potato, and onion are given.

Procedures

Because of inherent differences between these vegetables the procedures are not all the same.

Extraction: Distilled water is used as the extraction medium for dehydrated carrot, cabbage, sweet potato, and onion. The samples, ground to pass through a 2 mm. screen, are allowed to steep for 1 hour in distilled water at room temperature. Extraction of water-soluble colored materials reaches equilibrium under these conditions, as is shown for carrot and sweet potato in Table 1.

TABLE 1

Rate of Extraction of Color from 2 mm. Samples in Distilled Water at Room Temperature. Containers Swirled Every 30 Minutes.

$E^{2.5\%}_{2\text{ cm.}}$, 420 mμ

Period of Soaking	Optical Density [1]	
	Sweet Potato (Browned)	Carrot (Browned)
1 hr.	0.143	0.209
2 hr.	0.146	0.213
3 hr.	....	0.209
4 hr.	0.145	0.209

[1] Average for duplicate determinations.

Shaking proved undesirable for extraction of these vegetables; constant values were not reached for carrot samples (nor for sweet potato samples) after 4 hours' shaking, presumably because water-insoluble pigments were thrown into suspension by the shaking.

Water was used as the solvent in extraction procedures used by Campbell and Kilpatrick *(2)* for dehydrated white potato and by Burton *(1)* for potato powder. However, the turbidity of aqueous potato extracts is relatively high in comparison with the small amount of browning which is acceptable in dehydrated potato. Solutions containing organic solvents were found to extract without developing turbidity, but larger amounts of organic solvents in the mixture seriously retard the extraction of the water-soluble brown pigment. The best compromise between speed of extraction and reduced turbidity for white potato was found with 55 volume percent ethyl alcohol-water solution. Table 2 shows that extraction of soluble colored materials reaches equilibrium in the two-hour shaking period. Extraction mixtures such as this could not be used for the other vegetables in this series because of the extraction of excessive amounts of fat-soluble pigments such as the carotenoids.

TABLE 2

Rate of Color Extraction from 40 Mesh Browned Dehydrated White Potato in 55 Volume-% Alcohol-Water Solution.

$E^{5\%}_{2\text{ cm.}}$ 390 mμ

Period of Shaking	Optical Density
1/3 hr.	0.463
1 hr.	0.517
2 hr.	0.530
4 hr.	0.533

Clarification: Crude water extracts of dehydrated onions are clarified simply by repeated filtration through qualitative filter paper. This procedure is not effective for dehydrated carrot, cabbage, and sweet potato; extracts of these vegetables are clarified by passage through Seitz sterilizing filter pads.

Seitz pads absorb a small amount of color from the first extract passed through them (Table 3). Possible error is avoided by pretreating new pads with an

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TABLE 3

Absorption of Colored Materials from Successive Portions of Extracts Passed through Seitz Filter Pads.

$E^{2.5\%}_{2\text{ cm., }420\text{ m}\mu}$

Effluent Portion	Carrot (Browned)		Cabbage (Unbrowned)	
	Volume of Effluent Portion	Optical Density	Volume of Effluent Portion	Optical Density
1	25 ml.	0.291	40 ml.	0.088
2	25 ml.	0.325	37 ml.	0.099
3	25 ml.	0.340	33 ml.	0.109
4	25 ml.	0.357	33 ml.	0.115
5	25 ml.	0.362	35 ml.	0.114

extract of the undeteriorated vegetable prior to passage of the sample being tested. The Seitz pads also usually increase the pH of the filtrate. The amount is reasonably constant among pads from a given lot but is variable among pads from different lots. pH values ranging from 6.0 to 7.5 have been observed. Since the transmittancies are affected by pH (Figure 1), extracts of the vegetables clarified by Seitz pad filtration are adjusted to pH values at which their transmittancies are insensitive to change in pH. These pH adjustments are kept as small as possible because of increase in turbidity with decreasing pH. The same Seitz pad may be used for as many as 4 samples (one pretreatment); use is limited by decreasing rate of filtration and/or increasing turbidity of effluent.

Measurement of Light Transmittancy: Light transmittancies of the clarified extracts are measured with color filters peaked at 390 or 420 mμ; these transmittancies are expressed in terms of the optical density, log I_o/I. In referring to the optical density the symbol E is used with a superscript and two subscripts which represent, respectively, the extract concentration, cell length, and color filter used. Thus $E^{5\%}_{2\text{ cm. }390\text{ m}\mu}$, refers to the optical density of an extract prepared with 5 grams of material per 100 ml. of solvent, with transmittancy measured in a 2-cm. cell at 390 mμ. The difference between optical density values for samples before and after storage is taken as a measure of degree of browning during storage.

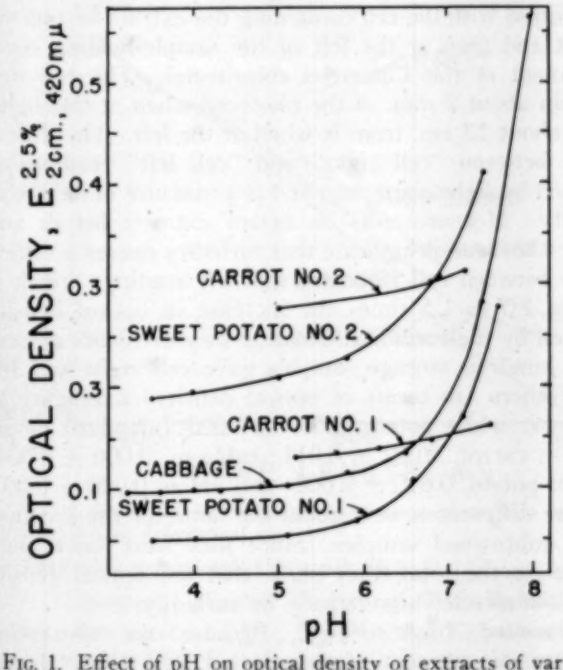

FIG. 1. Effect of pH on optical density of extracts of various dehydrated vegetables. Cabbage, sweet potato No. 1, and carrot No. 1: not browned. Sweet potato No. 2 and carrot No. 2: browned.

The 420 mμ color filter is used for carrots, cabbage, and sweet potato. The 390 mμ filter is used for white potato and onion (for which less browning is acceptable), because of the higher optical density values obtained, although satisfactory results are also obtained with the 420 mμ filter. Light absorption in extracts from all these vegetables increases markedly and continuously with decreasing wave length over the range of 600 mμ down to 350 mμ. This is shown for white potato in Figure 2.

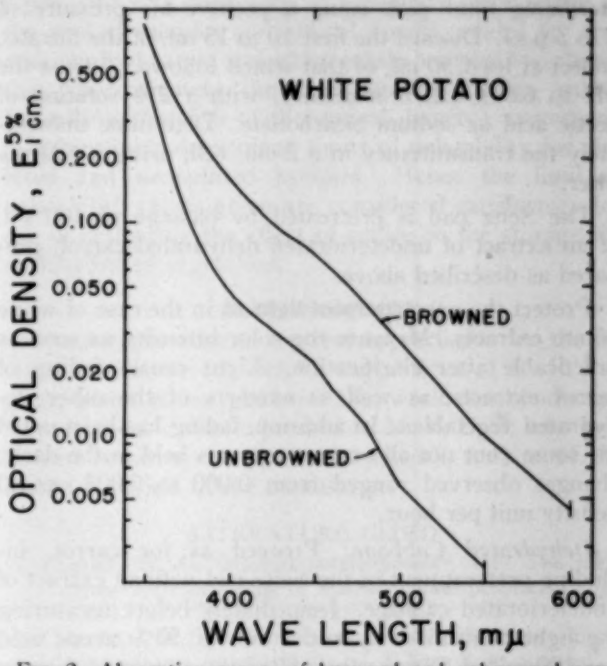

FIG. 2. Absorption curves of browned and unbrowned nonsulfited dehydrated white potato. Optical density values determined using 1 and 10 cm. cells, equivalent 1 cm. values being calculated from the 10 cm. values.

A Lumetron photoelectric colorimeter Model 402E was used in this Laboratory for routine measurements. Absorption spectra were determined with a Beckman quartz spectrophotometer.[b]

Dehydrated White Potato: Grind the sample to pass through a screen with openings of about 2 mm. Regrind a representative portion to pass through a U. S. Standard 40-mesh sieve. Weigh a 10-gram (MFB, moisture-free basis) sample into a 500-ml. glass-stoppered bottle and cover with 200 ml. of 55 volume-% alcohol. Fasten the stopper and shake with a mechanical agitator for 2 hours. Filter one funnelful of crude extract twice through an 18½ cm. Whatman No. 3 filter paper, covering the funnel to prevent evaporation of alcohol. Measure the transmittancy of the clarified extract in a 2-cm. cell, using a 390 mμ filter.

Although the color is stable for several hours in the dark, fading has been observed for extracts held in the

[b] In this paper mention of commercial names of equipment does not constitute a recommendation over other comparable products not named.

diffuse light of a north window. For example, the optical density of one extract changed from 0.340 to 0.260 during 6 hours' exposure. Hence, the extract should be protected from light by suitable means, such as enclosing the flasks in cardboard cylinders. An enclosed stirrer may be used instead of the mechanical shaker.

Dehydrated carrot: Grind the sample to pass through a 2-mm. screen. Weigh a 5-gram (MFB) portion into a 250-ml. Erlenmeyer flask and cover with 200 ml. of distilled water. Extract for 1 hour, swirling at the beginning, middle, and end of the period. Filter the crude extract through a 24-cm. Whatman No. 12 fluted filter paper; then refilter through a pretreated Seitz sterilizing filter pad, using a positive air pressure of 2 to 5 p.s.i. Discard the first 10 to 15 ml. of the filtrate; collect at least 50 ml. of that which follows. Adjust the pH to 6.6 ± 0.2, if necessary, with a 2% solution of acetic acid or sodium bicarbonate. Determine immediately the transmittancy in a 2-cm. cell, using a 420 mμ filter.

The Seitz pad is pretreated by passage of 100 ml. of an extract of undeteriorated dehydrated carrot, prepared as described above.

Protect the extracts from light as in the case of white potato extracts. Measure the color intensity as soon as practicable after clarification. Light causes fading of carrot extracts, as well as extracts of the other dehydrated vegetables. In addition, fading has been noted for some (but not all) carrot extracts held in the dark; changes observed ranged from 0.000 to 0.003 optical density unit per hour.

Dehydrated Cabbage: Proceed as for carrot, including pretreatment of the Seitz pad with an extract of undeteriorated cabbage. Immediately before measuring the light transmissivity add 1 ml. of 50% acetic acid per 100 ml. of filtrate; the pH of the extract is thereby adjusted to about 3.9. Measure transmittancy in a 2 cm. cell using a 420 mμ filter.

Dehydrated Sweet Potato: Proceed as for carrot, including pretreatment of the Seitz pad with extract of undeteriorated sweet potato. Immediately before determining transmittancy, adjust the pH of the clarified extract to 4.5 ± 0.2; this may be accomplished by addition of 1 ml. of a sodium acetate-acetic acid buffer per 100 ml. of extract, using a buffer solution containing 25 grams of acetic acid and 32 grams of sodium acetate trihydrate per 100 ml. Measure transmittancy in a 2-cm. cell at 420 mμ.

Dehydrated Onion: Extract a 4-gram (MFB) sample, ground to pass through a 2-mm. screen, with 200 ml. of distilled water, swirling the flask at the beginning, middle, and end of extraction, using an extraction period of 1 hour. Filter the crude extract through a 24-cm. Whatman No. 12 fluted filter paper; return the filtrate to the filter after 10 to 15 minutes and again after 15 to 30 minutes, measured from the start of filtration. As soon as sufficient filtrate is collected, measure the transmittancy of the extract in a 2-cm. cell using a 390-mμ filter. A 420-mμ filter also gives satisfactory results.

Discussion

As previously reported *(7)*, good reproducibility and good correlation with visual color scores were obtained by the above procedures. Standard deviations for 8 replicate determinations on each vegetable, both browned and unbrowned, ranged from 1.3 to 3.0% of the optical density.

Typical optical density values are given in Table 4 for freshly dehydrated vagetables and for the same vegetables after browning to arbitrarily selected "limits of palatability." These limit-of-palatability values represent the subjective appraisal of our panel.

TABLE 4

Typical Optical Density Values for Freshly Dehydrated Vegetables and for Vegetables Browned to Arbitrary "Limits of Palatability."

Commodity	Conditions	Optical Density	
		Freshly Dehydrated	Browned to Arbitrary "Limit of Palatability"
White Potato	$E^{5\%}_{2\text{ cm., }390\text{ m}\mu}$	0.140	0.300
White Potato sulfited	$E^{5\%}_{2\text{ cm., }390\text{ m}\mu}$	0.110	0.300
Carrot	$E^{2.5\%}_{2\text{ cm., }420\text{ m}\mu}$	0.140	0.680
Carrot sulfited	$E^{2.5\%}_{2\text{ cm., }420\text{ m}\mu}$	0.070	0.680
Cabbage sulfited	$E^{2.5\%}_{2\text{ cm., }420\text{ m}\mu}$	0.110	0.300
Sweet Potato	$E^{2.5\%}_{2\text{ cm., }420\text{ m}\mu}$	0.050	.... [1]
Onion	$E^{2\%}_{2\text{ cm., }390\text{ m}\mu}$	0.180	0.600

[1] Browning of the sweet potato proceeded so slowly that the limit of palatability with respect to browning was not reached.

Measure of Degree of Clarity: To be sure that errors were not caused by turbidity, light transmission was measured with the cell containing the extract first at the right and then at the left of the sample-holding compartment of the Lumetron colorimeter. The cell was within about 2 mm. of the photocell when at the right, and about 13 cm. from it when at the left. The difference between "cell right" and "cell left" readings is caused by light scattering, and is a measure of degree of clarity. Measurements on carrot extracts before and after clarification indicate that turbidity causes a difference between cell right and cell left readings which is about 1.0 to 1.5 times the increase in optical density caused by the turbidity. Routine measurements on several hundred storage samples gave cell right and left differences (in terms of optical density) averaging as follows: white potato, 0.007 ± 0.003 (standard deviation); carrot, 0.022 ± 0.011; cabbage, 0.006 ± 0.004; sweet potato, 0.010 ± 0.002; and onion, 0.020 ± 0.007. These differences were about the same for the browned and unbrowned samples. Since they were small compared to the total, they show that the optical density was not affected significantly by turbidity.

Standard Light Filter: Because the absorption spectra of extracts of dehydrated vegetables change rapidly in the region in which the color is measured, there has been need for a standard for comparison of

results obtained with different colorimeters, and also for use in checking day-to-day variation of the same colorimeter. A color filter has been found (Corning No. 4303) which meets this need. The absorption curve of this filter is similar to that of extracts of the dehydrated vegetables at the wave lengths used for color measurement (Figure 3). With this standard filter repeated day-to-day optical density values of 0.314 ±0.009 and 0.204 ± 0.007 have been obtained for the 390 and 420

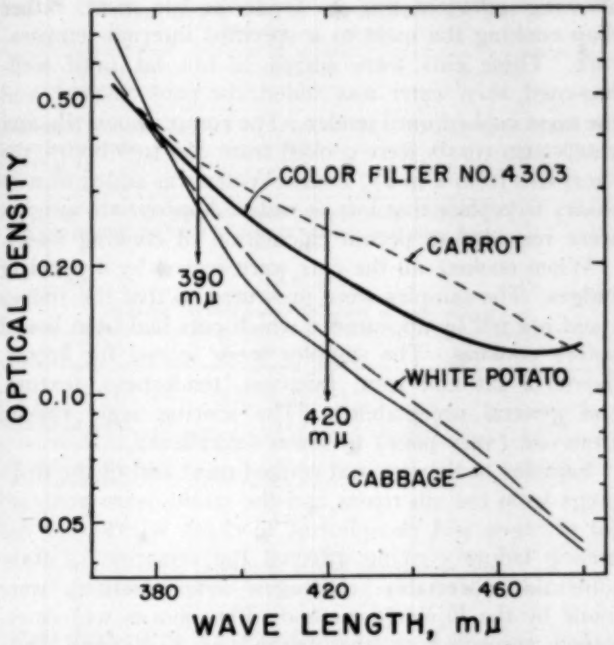

FIG. 3. Comparison of shape of absorption curves at about 390 and 420 mμ. Corning color filter No. 4303 and extracts of browned sulfited dehydrated vegetables. White potato: $E^{5\%}_{2\text{ cm.}}$; carrot and cabbage: $E^{2.5\%}_{2\text{ cm.}}$

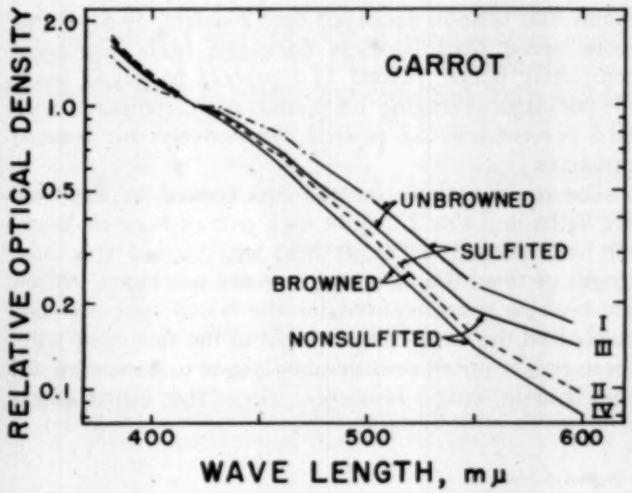

FIG. 4. Effect of sulfite and browning on absorption spectra for extracts of dehydrated carrot.

Sulfited: I, unbrowned, 1500 p.p.m. SO_2, $E^{2.5\%}_{2\text{ cm., }420\text{ mμ}}$ = 0.124 (Lumetron); II, after 17 days at 49° C., residual SO_2 content, 700 p.p.m., $E^{2.5\%}_{2\text{ cm., }420\text{ mμ}}$ = 0.259. Nonsulfited: III, unbrowned, $E^{2.5\%}_{2\text{ cm., }420\text{ mμ}}$ = 0.140: IV. after 9 days at 49° C. $E^{2.5\%}_{2\text{ cm., }420\text{ mμ}}$ = .308. Relative optical density = optical density at a given wave length/optical density at 420μ.

mμ filters, respectively, the instrument being set to zero optical density with a 2-cm. cell of distilled water in the sample holder.

Effect of Sulfite: Sulfite exerts some effect on the absorption spectra of the extracts (Figure 4). This effect was observed in pronounced form by Stadtman, Barker, Mrak, and Mackinney *(10)* for dried apricots containing 800 and 20,000 p.p.m. SO_2; these authors concluded that measurements of degree of darkening by photometric procedures would be valid only for fruit of the same initial sulfite content. In the case of dehydrated vegetables the effect of sulfite on the absorption spectra is much smaller, perhaps because of the much lower sulfite content (seldom over 1000 to 1500 p.p.m. SO_2). Comparison of subjective color scores of the cooked samples and optical density values for several hundred carrot and white potato samples has shown that approximately the same optical density values (usually within 5% of the optical density) correspond to subjectively determined limits of palatability for sulfited and nonsulfited samples. Hence the limit of palatability values given are considered satisfactory for use in evaluating the effect of sulfite on the storage life of dehydrated vegetables.

Acknowledgment

The authors wish to express appreciation for the interest and numerous helpful suggestions of R. R. Legault, under whose guidance this work was conducted. Appreciation is also expressed to M. F. Pool, who performed some of the preliminary work, and to H. K. Burr for review of the manuscript.

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Effect of Boning on Cooking Losses and Palatability of Beef[a]

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Tests were made to determine the effect of boning beef on space required for frozen storage, on cooking losses, and palatability of the resulting product. Bone-in packaged beef required 34.8% more frozen storage space than boneless packaged beef. The cooking time, cooking losses, palatability, and nitrogen and phosphorus content were very similar for the boned and bone-in cuts, the differences being too small to be significant.

Many people feel that beef cooked with the bone in has a flavor superior to a similar cut boned before cooking. However, one of the suggestions usually made for saving freezer space is to bone meat cuts before freezing. The work reported below was designed to test whether or not boning beef before cooking affected the palatability and the cooking losses, and to compare locker space required to store the comparable boneless and bone-in cuts.

Cutting, boning and packaging tests were made on two commercial grade carcasses, two good grade carcasses and four choice grade carcasses. One side of each carcass was cut and packaged bone-in, excepting the fore and hind shanks which were boned. The remaining side of each carcass was completely boned and packaged.

Packaging was done by using a cellophane inner wrap and a No. 30 weight kraft paper overwrap. The packages from each side were frozen and then packed in a container to determine space requirements.

Paired cuts from the right and left sides of one animal were used for cooking and palatability tests. Cuts included 2 pairs of rib roasts, 2 pairs of club steaks, 4 pairs of porterhouse steaks, 4 pairs of sirloin steaks, 2 pairs of rump roasts, 2 pairs of chuck rib roasts, 2 pairs of chuck arm roasts and 4 pairs of short ribs. All cuts were wrapped in cellophane and frozen until the day before they were to be cooked. As needed, they were thawed in a refrigerator. Just before cooking, one cut from each pair was boned.

The rib roasts were cooked in an oven at 149° C. (300° F.) to an internal temperature of 58° C. (136.4° F.) (rare). The steaks were oven-broiled to an internal temperature of 58° C. (136.4° F.) at an oven temperature of 190.5° C. (375° F.). The rump and chuck roasts and short ribs were cooked by moist heat. The cooking time for these was fixed in terms of allowing sufficient time to tenderize the meat, rather than cooking the meat to a specified internal temperature. These cuts were seared in hot fat until well-browned, then water was added, the pans covered, and the meat cooked until tender. The rump, chuck rib, and chuck arm roasts were cooked from 3½ to 4 hours, the short ribs from 2 to 2½ hours. Water was added as necessary to replace that lost as steam. Appropriate weights were recorded to permit calculation of cooking losses.

When cooked, all the cuts were scored by a panel of judges. The samples were presented so that the judges could not tell by appearance which cuts had been boned before cooking. The samples were scored for aroma, flavor of fat and lean, juiciness, tenderness, texture, and general acceptability. The scoring scale ranged from one (very poor) to seven (excellent).

Samples of the raw and cooked meat and of the drippings from the rib roasts and the steaks were analyzed for nitrogen and phosphorus, to check whether or not boning before cooking affected the retention of these minerals appreciably. Nitrogen determinations were made by the Kjeldahl method. Phosphorus was determined according to the method of Fiske and Subbarow *(1)*.

Results

Table 1 summarizes the data relative to cutting yields and space requirements of the eight carcasses. It was found that bone-in packaged beef required 34.8 percent more space than boneless packaged beef. Carcasses completely boned yielded 71.4 percent packaged meat, the packages averaging 1.8 pounds each, compared with 88.6 percent and 2.7 pounds, respectively, for bone-in carcasses.

The cooking times for the cuts cooked by dry heat are listed in Table 2. Since each pair of bone-in, bone-out cuts cooked by moist heat was cooked the same length of time, the data for these are not listed. When the cooking time required for the boned cuts was calculated on the basis of the weight of the raw meat only, these cuts required considerably longer to cook than did the bone-in cuts. However, since the factor being

[a] Presented before the Tenth Annual I. F. T. Convention, Chicago, Illinois, May 22, 1950.

[b] Journal article No. 1125 from the Michigan Agricultural Experiment Station.

TABLE 1

Boneless and bone-in packaging of beef

Carcass Grade	Boneless				Bone-In			
	Side Wt. Lbs.	Percent Pkgd.	Pounds Per Pkg.	Cubic Feet Required	Side Wt. Lbs.	Percent Pkgd.	Pounds Per Pkg.	Cubic Feet Required
Commercial	181.0	72.2	1.6	3.68	180.5	91.2	2.7	4.81
Commercial	166.0	71.6	1.6	3.43	162.5	91.0	2.3	4.68
Good	249.5	73.1	1.9	5.21	233.0	89.8	2.9	7.41
Good	218.5	71.5	1.7	4.93	220.0	89.2	2.6	6.67
Choice	308.5	71.6	2.0	6.80	306.5	85.3	3.1	8.17
Choice	305.0	71.3	1.8	6.27	303.5	86.8	2.7	8.22
Choice	292.5	72.2	1.9	5.76	286.0	89.8	2.6	8.10
Choice	307.0	68.6	1.7	5.72	315.0	88.1	3.1	8.27

measured was the rate of heat penetration into the meat rather than the bone, it seemed more logical to compare the figures calculated on the same basis, that is, weight of meat plus bone. From this standpoint, the differences in cooking times between the boned and unboned cuts were not significant.

Table 3 gives the percent cooking losses for the various cuts. Since the bone would be expected to contribute little if any to the losses during cooking, again it seemed more logical to compare the losses calculated from the weight of both meat and bone. Here also the differences between boned and unboned cuts were not significant.

The chuck pot roasts showed higher cooking losses than the rump or short ribs, the differences being highly significant ($p < 0.01$). Two factors undoubtedly contributed to this: (1) the rump contained much less fat than the chuck; (2) the short ribs were cooked for a shorter period of time than the chuck.

The average scores for flavor of fat and lean, juiciness, tenderness, and general acceptability are given in Table 4. In all the cuts, the differences between those cooked with and without bone were too small to be significant. However, the differences in tenderness between the club, porterhouse and sirloin steaks, and in flavor of fat and juiciness between the rump roasts, chuck roasts and short ribs were highly significant, indicating that differences between different cuts cooked by the same method were much larger than differences arising from whether or not the bone was removed before cooking.

Table 5 gives the nitrogen and phosphorus contents of the porterhouse and sirloin steaks, and the rib roasts. Boning seemed to make very little difference in the retention of these two minerals during cooking by dry heat.

TABLE 2

Average cooking time for cuts cooked by dry heat (minutes per pound)

Cut	Bone in	Bone out	
		A	B
Club steak	38.3	53.1	37.7
Porterhouse steak	24.9	27.6	22.8
Sirloin steak	22.4	25.4	20.2
Rib roasts	31.2	42.9	30.3

A—calculated on basis of weight of raw meat only.
B—calculated on basis of weight of raw meat plus bone.

TABLE 3

Average cooking losses (percent of raw weight)

Cut	Bone in	Bone out	
		A	B
Club steak	14.8	16.5	11.9
Porterhouse steak	14.1	12.6	10.4
Sirloin steak	14.7	18.5	14.7
Rib roast	18.1	21.1	14.8
Chuck arm roast	36.4	37.7	33.5
Chuck rib roast	31.5	38.5	30.7
Rump roast	28.0	37.2	29.7
Short ribs	26.3	31.5	25.2

A—losses calculated on basis of weight of raw meat only.
B—losses calculated on basis of weight of raw meat plus bone.

Summary

Beef carcasses completely boned and packaged require significantly less frozen storage space and can provide smaller sized packages than comparable carcasses packaged with little or no boning.

The cooking time, cooking losses, palatability, and nitrogen and phosphorus content were very similar for bone-in and boneless beef cuts, the differences being too small to be significant. The major differences found were between different cuts cooked by the same method. The chuck roasts had the highest cooking losses of any of the braised cuts. The club steaks were the most tender and the sirloin steaks the least tender of the three kinds of steaks. The rump roasts received lower scores for flavor of fat, while the short ribs were given high scores for juiciness among the braised cuts. The lower juiciness scores for rump and chuck may be a reflection of the longer cooking time for these cuts.

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TABLE 4

Average palatability scores (maximum = 7)

Cut	Flavor-lean		Flavor-fat		Juiciness		Tenderness		Acceptability	
	Bone in	Bone out	Bone in	Bone out	Bone in	Bone out	Bone in	Bone out	Bone in	Bone out
Club steak	6.7	6.4	5.7	5.8	6.4	6.6	6.4	6.2	6.1	6.2
Porterhouse steak	6.5	6.5	5.9	6.0	6.3	6.6	5.9	5.9	6.2	6.2
Sirloin steak	6.1	6.0	5.5	5.6	6.2	5.7	5.6	5.6	5.8	5.8
Rib roast	6.3	6.6	6.2	6.3	5.8	6.3	6.2	6.4	6.1	6.4
Chuck arm roast	5.6	6.0	6.0	6.2	3.7	4.1	5.3	5.4	5.3	5.6
Chuck rib roast	5.9	5.7	5.9	6.0	4.6	4.8	5.8	5.8	5.4	5.6
Rump roast	5.6	5.3	5.2	5.4	3.8	3.5	5.9	6.1	5.0	4.8
Short ribs	5.9	5.9	6.1	5.9	5.1	5.1	5.4	5.1	5.4	5.3

TABLE 5

Average nitrogen and phosphorus contents of steaks and roasts

Cut	Nitrogen				Phosphorus					
	Raw [1]		Cooked [1]		Raw [2]		Cooked [2]		Drippings [3]	
	Bone in	Bone out	Bone in	Bone out	Bone in	Bone out	Bone in	Bone out	Bone in	Bone out
Porterhouse steak	3.03	3.11	4.36	4.03	171	177	211	202	70	85
Sirloin steak	3.14	3.16	3.63	3.61	185	182	190	198	15	21
Rib roast	3.26	3.04	4.08	4.11	183	183	187	186	—	22

[1] Percent of wet weight.
[2] Milligrams per 100 g. wet weight.
[3] Milligrams per 100 g. raw sample, wet weight.

Color Measurement of Tomato Purees[a]

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(Received for publication, May 29, 1950)

An objective method of measuring tomato puree color is described. The method consists of (a) determination of color specifications with a Hunter Color-Difference Meter and (b) interpretation of specifications on a special chromaticity diagram.

Color is one of the commonly used criteria of tomato quality. Such terms as "good red color" or "well-colored" are often used to describe this quality characteristic but in most cases the colors referred to neither exist in the form of permanent standards nor are described in terms of a color measurement system. A system for specifying tomato colors is needed so that color description may be more effective.

This problem has been recognized by others and studies related to the subject have been reported by Davis *(2)*, Carrasco *(1)*, Kramer and Smith *(4)*, MacGillivray *(5, 6, 7, 8)*, Mitchell *(10)*, McCollum *(9)*, Taxner *(13)* and Vogele *(14)*. None of the methods proposed has been widely used because they either were slow and expensive, or the resulting data were not correlated with appearance, or the data were not readily reproducible. The writer *(15)* recently described a method involving the Color-Difference Meter developed by Hunter *(3)*. This method appears to have overcome most of the disadvantages of older methods. The method is reviewed and its application to two tomato color specification problems is illustrated.

Color Specification

The Color-Difference Meter specifies color directly in terms of L, a_L and b_L. As applied to tomato purees, these units measure lightness, redness and yellowness, respectively. In order to simplify interpretation, the color dimensions were plotted on chromaticity diagrams which show the location of certain similar colors in terms of Munsell specifications.

The Munsell system of color specification has been fully described by Nickerson *(12)*. The following discussion is intended only to introduce the basic idea of the system as related to the present problem. Psychologically, color has three attributes. These attributes are hue, value and chroma. Hue indicates whether the color is red, yellow, green, etc., value describes the lightness of the color, while chroma indicates the strength of the color or its relative difference from a gray of the same value. Relationships of the three attributes can best be shown in the three dimensional figure (Figure 1) known as the Munsell color solid. In the Munsell color solid five hues are arranged around the circumference of the solid and each hue is divided into twenty equally spaced intervals giving a total of 100 hue steps. Chroma is measured along radii extending outward from the axis of the figure and this dimension is also divided into equally spaced steps.

[a] Presented before the Tenth Annual IFT Convention, Chicago, Illinois, May 23, 1950.

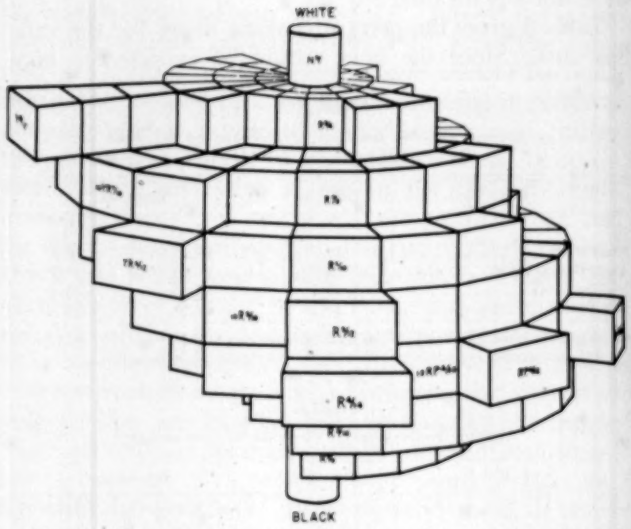

FIG. 1. Diagrammatic representation of the color solid used in the Munsell system. From Nickerson, Bull. Am. Ceram. Soc., **27**, 51 (1948).

The vertical axis of the figure is the value scale which extends from black through a series of grays to white.

A horizontal cross section through the color solid reveals all colors of a given lightness (Figure 2), while a vertical cross section exposes all colors of a given hue on one side of the axis and all colors of the complementary hue on the opposite side of the axis (Figure 3).

With appropriate formulas the Munsell hue and chroma loci may be converted to L, a_L and b_L. The

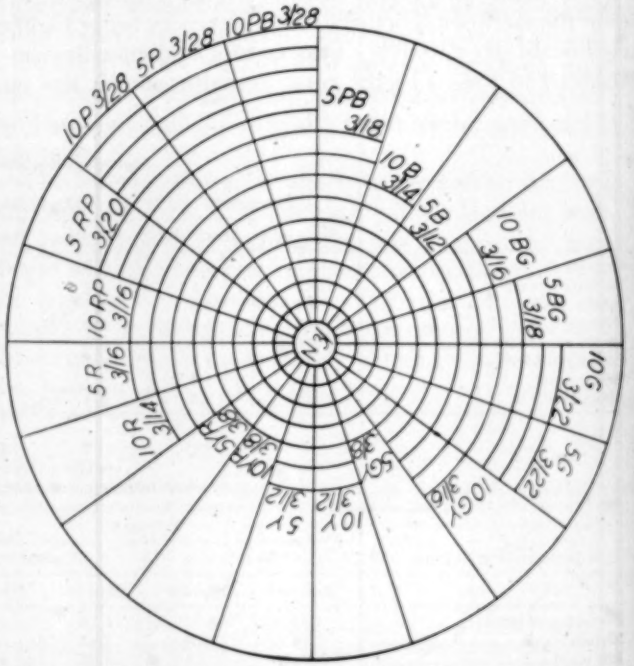

FIG. 2. Horizontal cross section of the Munsell color solid at value 3/. The plane exposes all colors at the designated lightness level.

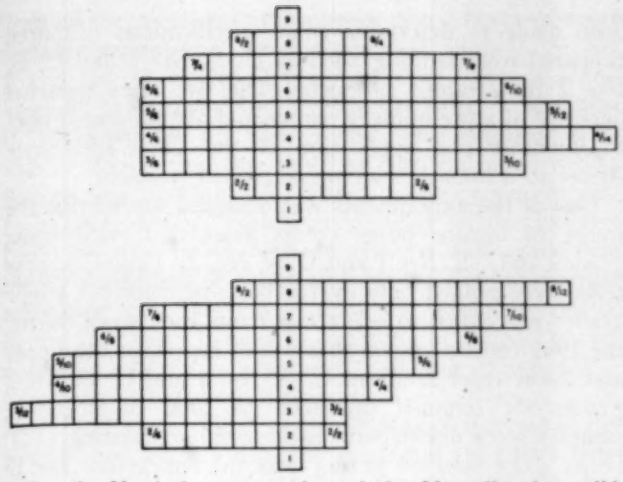

FIG. 3. Vertical cross section of the Munsell color solid showing complementary hues for red and blue-green, yellow and purple-blue. Each plane exposes two complementary hues and the axis of the solid. From Nickerson, Bull. Am. Ceram. Soc. 27, 51 (1948).

Munsell renotation loci given by Newhall, Nickerson and Judd *(11)* for value 3/ are shown in terms of a_L and b_L in Figure 4. The spacing of the hue and chroma steps is less uniform in terms of a_L and b_L than in terms of the Munsell units shown in Figure 2. It should be understood that the relationship shown in Figure 4 is true only when L is 25.6. Position of constant hue and chroma lines change as lightness changes because of the configuration of the color solid. Data in terms of a_L and b_L for Munsell renotation hue and chroma loci at lightness levels applicable to most tomato products have been given by the writer *(15)*.

Colors of tomato purees varied in lightness (L) between 22 and 29 thus the level shown in Figure 4 was intermediate and accurate enough for comparison of the color specifications. Hues of tomato purees varied between 2.5YR and 7.5R while chromas varied from /6 to /8. The chromaticity diagram that has been used for tomato puree colors is shown in Figure 5. This diagram is an expansion of that part of the diagram in Figure 4 which is applicable to tomato puree colors. The range of hues and chromas shown in the diagram differ somewhat from those reported by MacGillivray (5). The difference is probably due in part to differences in sample illumination and viewing conditions.

Color specifications obtained from the Color-Difference Meter in terms of a_L and b_L were plotted on the diagram, while the lightness reading was written beside the plotted point. Colors that plotted near the 2.5YR hue line were undesirable, while colors that plotted near the 7.5R hue line were highly desirable. Thus the hue of a puree was evaluated with respect to its proximity to one of the constant hue lines. If hue differences were equivalent to 0.3 of a unit on the a_L scale, observers noted differences in color. Colors that plotted near chroma /6 were weak and less desirable than those plotted near chroma /8 but differences in chroma had to be in excess of one-half step to be readily perceptible. One L unit represented the minimum perceptible change in lightness. As L declined in value, colors became darker and more attractive providing L

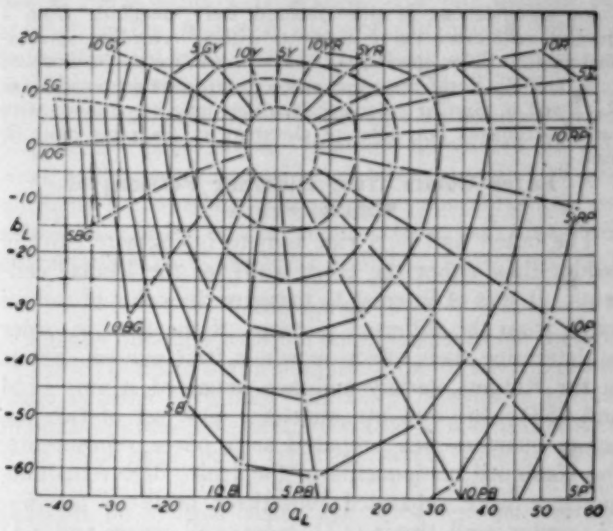

FIG. 4. Munsell renotations for value 3/ plotted in terms of Hunter's a_L and b_L. Note that colors exposed are the same as in Figure 2. Hue and chroma lines are less uniformly spaced when expressed in terms of a_L and b_L.

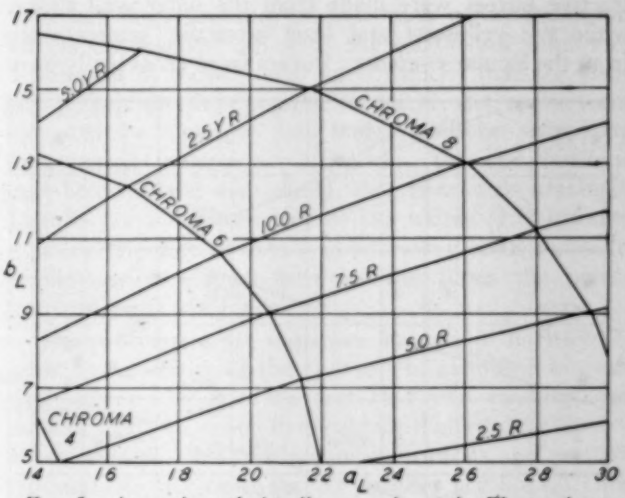

FIG. 5. A portion of the diagram shown in Figure 4 containing the hues and chromas commonly found in tomato purees. The central portion of this area is further enlarged to make the chromaticity diagram used for color plotting.

was above 23. Below 23, puree colors were objectionably dark. Minimum perceptible differences were based on the observations of persons experienced in grading tomato puree colors.

Sample Preparation and Color Measurement

All color measurements were made on tomato puree because such preparations were more uniform than raw or canned whole fruits. The purees were prepared from washed and quartered fruits that were macerated and carefully heated to 80° C. with constant stirring. When temperatures reached 80° C the fruits were passed through a Juice-X machine or a small horizontal, paddle-type extractor. The Juice-X machine was used for samples consisting of 15 to 20 fruit, while the horizontal extractor was used for larger samples. Purees were deaerated, cooled to room temperature and agitated to uniformly resuspend solids before being transferred to viewing cells. Viewing cells were glass cylinders 6.5 centimeters in diameter and 4 centimeters high with the base constructed of optically colorless glass.

Circuits of the Color-Difference Meter were initially balanced with a specially prepared, red porcelain enamel panel.

Color specifications of this standard were computed from a spectral reflectance curve obtained with a recording spectrophotometer. Color specifications were read directly in terms of L, a_L and b_L. Some color-difference meters contain circuits for R_d, a and b. Similar color specifications may be obtained with such instruments but different chromaticity diagrams must be used.

Puree Colors from Different Portions of Tomato Fruits

The outer wall of a ripe tomato is characteristically redder than either the cross walls, or the locular contents. It was of interest to measure the color of purees made from the different portions of the fruit in order to determine their effect on whole fruit purees. Fruits of the Rutgers variety and two unnamed strains (164 and 858) were cut into quarters. One set of two opposite quarters was prepared as a puree representing the combined components of the fruit. The remaining quarters were separated into three parts as follows: (1) outer wall tissue, (2) cross wall tissue and (3) locular contents. Each lot was processed as a puree and the resulting color measured. The results are shown in Figure 6. In each instance the reddest and most attractive purees were made from the outer wall tissue, while the yellowest and least attractive purees were from the locular contents. Purees from cross walls were

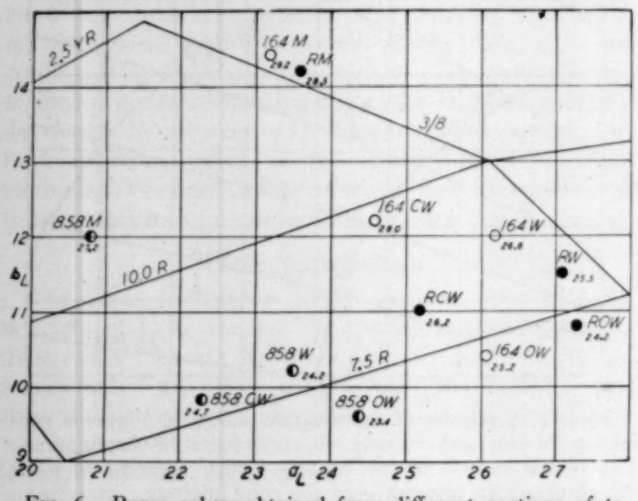

FIG. 6. Puree colors obtained from different portions of tomato fruits of the Rutgers (R) variety and two experimental strains (164 and 858). Colors of purees obtained from all parts of the fruit (W), outer walls (OW), cross walls (CW) and locular contents (M) are shown.

intermediate in color and appearance. In each case the purees from the combined portions of the fruit were intermediate in color between the outer and cross wall purees. While the color of purees from the outer walls of 858 were weaker than those of Rutgers and 164, 858 purees were redder and darker, thus more attractive. The cross walls of strain 164 fruits were substantially less red than those of either Rutgers or 858, and as a result the whole fruit puree of 164 was more yellow and less attractive than whole fruit purees from Rutgers and 858.

Puree Colors from U. S. No. 1 and U. S. No. 2 Fruits

MacGillivray *(8)* used a disc colorimeter to compare the color of tomatoes graded as "well-colored" and "fairly well-colored" but apparently no attempt has been made to determine color specifications of puree prepared from fruits graded as U. S. No. 1 and U. S. No. 2 for strained products. The two tests reported here are of a preliminary nature and are presented only to illustrate one application of the Color-Difference Meter to a tomato color measurement problem.

One of the experiments was designed to describe the range of tomato puree colors associated with fruits graded as U. S. No. 1 and U. S. No. 2. Fruits in these tests were graded only on the basis of color. A single grader was asked to select raw fruits each week during the 1949 tomato season that would represent the upper and lower color limits of U. S. No. 1 and U. S. No. 2 grades for strained products. A total of thirty-six samples were drawn with each sample consisting of 20 fruits. The selected grader was the supervisor for 15 to 20 inspectors employed at a single grading station, thus he probably had a better than average knowledge of grade limits. Purees were prepared soon after the samples were selected and the color of each puree was measured. Colors of purees obtained over a period of 9 weeks are shown in Figure 7.

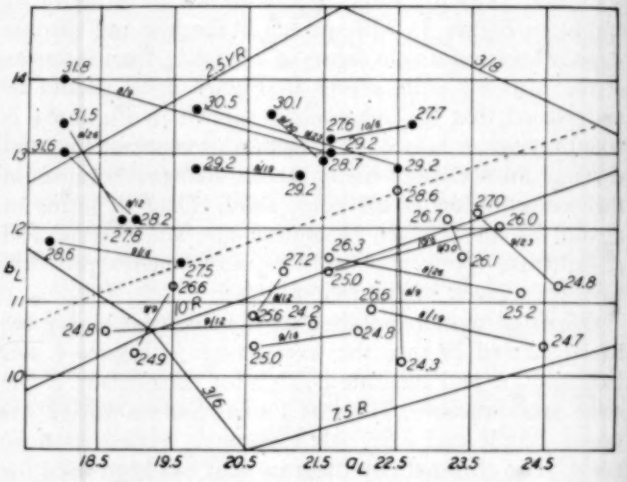

FIG. 7. Tomato puree colors obtained from fruits selected as representing the upper and lower color limits of U. S. No. 1 (open circles) and U. S. No. 2 (solid circles) fruits on 9 different dates. Points representing colors of the upper and lower limits of No. 1 and No. 2 fruits on each date are connected with lines. The date each pair of samples was collected is indicated. The upper limit of each grade is represented by the point nearest the 7.5R hue line. The dotted line represents the critical hue line separating U. S. No. 1 and U. S. No. 2 colors.

The number of samples drawn was limited but certain results were of particular interest. First, the grader was unable to repeatedly select fruit describing grade limits. On August 12, September 9 and September 30, fruits selected to represent the upper color limit of the U. S. No. 1 grade were inferior in color to fruits selected on August 16 and August 19 to represent the lower color limit of U. S. No. 1 grade. Similar results were noted in fruits graded as high or low U. S. No. 2 and on September 4 and September 12, all U. S. No. 2 fruits were so inferior in color that they could not be plotted on the chromaticity diagram used in Figure 7. Second, despite the grader's inability to repeatedly select grade limits, the puree colors from U. S. No. 1

fruits were superior to those from No. 2 fruits in every instance except one. In this one case, high U. S. No. 2 and low U. S. No. 1 fruits produced purees of essentially the same color. It appeared that a hue near 0.5YR separated the two grades. The estimated position of this critical hue line has been indicated with a broken line in Figure 7. Third, it was apparent that fruits were almost exclusively graded on the basis of hue. No association between chroma and grade was noted. Lightness of colors and grades were apparently associated but this correlation was probably related to the fact that changes in lightness of tomato purees normally tend to be associated with hue changes.

In view of the sharp demarcation of the hues of U. S. No. 1 and U. S. No. 2 grades, it was of interest to determine how closely the other inspectors at this station approximated the same color separation of the two grades. On each of three successive days in late September samples were drawn from the grading tables representative of each grader's U. S. No. 1 and U. S. No. 2 fruits. Fruits placed in the No. 2 bin, for reason other than color, were not included in the samples. A total of 90 samples were drawn representing U. S. No. 1 and U. S. No. 2 fruits from 45 loads of tomatoes graded by 15 different inspectors. Colors obtained from the different samples are shown in Figure 8 along with the critical, constant hue line described above. In every instance hues of the purees from U. S. No. 1 fruits were equal or superior to that indicated by the minimum established by the supervisor. In three instances fruits graded as U. S. No. 2 produced purees above the minimum established for U. S. No. 1 fruits. In general, grades for color given by regular inspectors were in good agreement with those described by the supervisor.

The two tests strongly suggest that color separation of the raw fruit grades was based on hue. While a marked association was apparent between hue and brightness, other data reported by the writer *(15)* suggest that this association may be less important than data in this test indicate. In both tests it was apparent that the chroma attribute had no influence on the manner in which fruits were graded. The fact that the inspector in the first test did not distinguish between fruits that yielded purees having weak and strong colors indicates that the percentages of U. S. No. 1 and U. S. No. 2 fruits may not provide an accurate index of processed puree colors. It should be pointed out that weak colors were experienced after a period of abnormally hot weather. Low pigment concentrations resulted in purees having weak colors but with hues characteristic of U. S. No. 1 fruits.

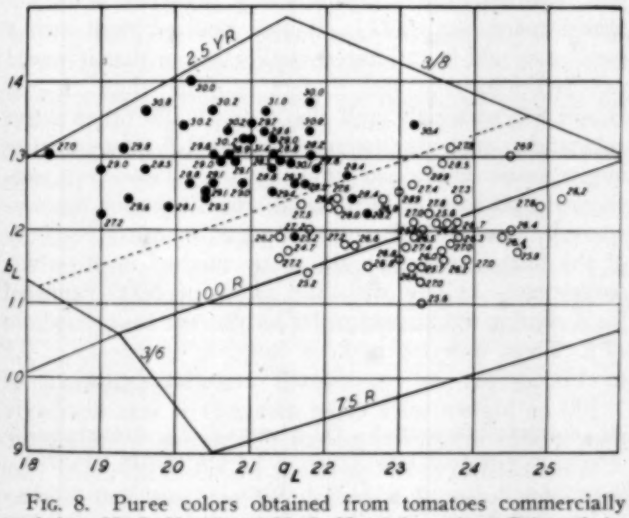

FIG. 8. Puree colors obtained from tomatoes commercially graded as U. S. No. 1 and U. S. No. 2 for color. Color of the purees from the No. 1 (open circles) and No. 2 (solid circles) fruits from each of 45 loads is shown. The dotted line represents the critical hue line separating the two grades.

Discussion

Results obtained with the color measurement method described indicate that a number of the disadvantages of other systems have been eliminated. Colors of several thousand purees have been measured in connection with tests that have been reported herein and in other studies. Most of these purees were also scored for appearance on a visual basis. The agreement between the two methods was excellent, regardless of the magnitude of the color difference. The Color-Difference Meter is capable of detecting differences in tomato puree colors not discernible to the average, experienced observer. With an understanding of how each color dimension affects appearance and with the established minimum differences in tomato color specifications that are visually perceptible, it is possible to quickly determine from chromaticity diagrams how colors will be scored by experienced observers. Moreover, the specifications may be compared with similar data previously obtained. Results previously reported by the writer *(15)* indicate that reproduction of data is good even though a considerable period elapses between the times the actual measurements are made.

The procedures are objective and are in no way related to the ability of the operator to match or to evaluate colors. Despite the fact that two readings are usually made in order to eliminate dial reading errors, it has been possible to obtain specifications and prepare chromaticity diagrams for 30 samples per hour. Thus the operation is not time consuming.

The basic principles of the present method have been used to measure colors of products other than tomato purees. Results of these tests have been equally satisfactory. It is believed that the Color-Difference Meter and chromaticity diagrams similar to the type described may prove immediately applicable to problems in the food processing industry where specification of relatively small color differences are desired.

Acknowledgments

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Vitamin B_{12}-Active Substances in Fish Products[a]

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The amount of vitamin B_{12}-active substances in fish flesh and organs, fish meals, stickwaters, condensed solubles and certain other materials was determined by microbiological assay using Lactobacillus leichmannii as test organism. The results of preliminary experiments dealing with the formation of vitamin B_{12}-active substances in fish stickwater by microbiological action are given.

Fish protein-containing by-products, particularly meals and condensed solubles, together with similar by-products of the meat packing industry have formed, until very recent times, the chief sources of vitamins of the "Animal Protein Factor" (A.P.F.) complex in livestock rations. A little over two years ago vitamin B_{12}, a substance which appears to fulfill the role of the extrinsic factor of Castle *(6)* in treatment of pernicious anaemia, was isolated *(14, 18)*. It was soon realized that vitamin B_{12} had marked A.P.F. activity when introduced in levels of from 0.6 to 3 μg per 100 gm. of A.P.F.-free chick diet *(12, 13)*. However, it still appears that, though vitamin B_{12} is undoubtedly an important component of the A.P.F., the latter also contains other growth factors the chemical nature of which is still in doubt. Thus a study of beef liver extracts using the counter current distribution method of separation has revealed the presence of two growth-stimulating factors for chicks which do not stimulate growth of *Lactobacillus leichmannii* in vitamin B_{12}-free medium *(3)*. Furthermore, certain commercial A.P.F. preparations which contain *Streptomyces* mash appear to possess growth factors for chicks which are not vitamin B_{12} and do not seem to occur in animal proteins *(21)*. The thesis that preparations of this type which contain vitamin B_{12} may act as indirect growth stimulators by suppressing undesirable bacteria in the digestive tract has been advanced *(22)*. Vitamin B_{12} and industrial A.P.F. preparations appear to have a number of functions in promoting proper growth in livestock, and among these the methionine and methyl donor sparing effects as well as the lipotropic action must be considered.

With the publication of some of the earlier work referred to above, it became obvious that it might be important not only to know the vitamin B_{12} content of fishery products, but that it might be wise to attempt some standardization and also possibly to increase the natural content by microbiological fermentation. The present paper describes experiments which have been carried out during the past year in connection with this problem *(10)*.

Distribution of Vitamin B_{12}-Active Substances in Fishery Materials

At the time this work was initiated, microbiological procedures for determining vitamin B_{12} were only just being made known and a method was selected which was reported to give results which paralleled those obtained using mice *(17)*. It was realized that such a method would have limited application in that it would not determine the A.P.F. factors other than vitamin B_{12} in fish materials, and also that, should other active substances such as desoxyribosides be present, they might cause fictitiously high results. However, tests showed that over 4600 times as much thymine desoxyriboside as vitamin B_{12} was required to cause response of the test organism in the assay method used, which corresponds closely with the value of 5000 reported for a similar test organism on a different assay medium *(7)*. Thus, unless present in amounts in excess of 0.5 to 1.0 μg per ml. of diluted material (dilutions of 1:100 or higher were often assayed) it was not likely that serious interference from this source would result.

In the isolation of anti-pernicious anaemia concentrates from liver some degree of proteolysis had been employed in order to free the vitamin *(20)*. Papain was used as proteolytic agent in the present work since the preparation used (from Nutritional Biochemicals Corp.) contained much smaller amounts of vitamin B_{12}-active substances than did an available sample

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of trypsin 1:300 from the same source. Recently, ordinary pancreatin has been used for this purpose (3). Cysteine hydrochloride (1.3 g.) was dissolved in 10 ml. of water, 1 g. of papain added and the mixture was incubated for 1 hr. at 37° C. to activate the enzyme. Blended fish muscle or organs were suspended in from 1 to 3 volumes of water, activated papain added (10 mg. per g. of wet tissue), the mixture (pH 7.0) was covered with a layer of toluene and digested for 1 day at 37° C. with occasional shaking. Fish meals (10 g.) were suspended in water (usually 100 ml.) and the suspensions held at 0° C. for one day to soften it. Activated papain (500 mg.) was then added and the sample incubated under toluene for 2 days. After incubation the samples were made to a known volume, filtered, and aliquots were taken for dilution before assaying. In cases where it was not possible to carry out assays of materials soon after digestion, the hydrolysates were frozen and stored at —20° C. With the organism and medium employed *(17)* best results were obtained when the materials were assayed in dilutions corresponding to the comparatively narrow limits of 0.01 to 0.04 mμg of vitamin B_{12} per ml.

The results of assays carried out on a variety of fishery materials are given in Table 1. For convenience they have been expressed as mμg of vitamin B_{12} per gram of dry, fat-containing materials. In considering these results consideration should be given to the fact that, should significant amounts of vitamin B_{12a} have been present in any of the materials, low results would have been obtained because *L. leichmannii* respond only 20 percent as much to vitamin B_{12a} as to vitamin B_{12} *(24)*. The samples analyzed were reasonably fresh, but no attempt was made to ascertain whether significant decreases in vitamin B_{12} occur post mortem.

Kidney was especially rich in vitamin B_{12}-active substances, while spleen, stomach, pyloric caeca, eggs and, to a lesser extent, milt were good sources of the vitamin. With the exception of a single sample of very oily dogfish liver examined, all livers contained marked but variable amounts of vitamin B_{12}. The muscle tissue of white fleshed fish and halibut heads were very poor sources of the vitamin.

Condensed fish solubles were generally good sources of vitamin B_{12} and, with the exception of three samples (one each from whale and dogfish and a sample of undetermined origin), values from 200 to 670 mμg per gram were obtained. Stickwaters resulting from salmon offal reduction gave values comparing favorably with the best condensed solubles. Fish meals prepared from salmon offal reduction contained considerably less vitamin B_{12} than did two samples prepared from whole Pacific herring. The single sample of clams tested proved to be an excellent source.

The possibility that fishery materials contain growth factors for chicks other than vitamin B_{12} is supported by the finding that chicks respond to condensed solubles to a greater extent than can be accounted for by their vitamin B_{12} content as determined by microbiological assay with either *Euglena gracilis (8)* or *Lactobacillus lactis (2)*.

Separation of Vitamin B_{12}-Active Substances in Fishery Materials by Filter Paper Chromatography

Our preliminary work on this subject has been of a qualitative nature. Vitamin B_{12} (from Nutritional Biochemicals Corp.), thymine desoxyriboside and guanine desoxyriboside [prepared by Schindler's modification *(16)* of Klein's *(9)* method] were chromatographed at 20° C. for 16 hours *(23)*. The Rf values found were as follows and are within the range given by Winsten and Eigen *(23)* and Smith and Cuthbertson *(19)*: Vitamin B_{12}, double zone with Rf values of 0.03 and 0.06; guanine desoxyriboside, Rf 0.28-0.32 and thymine desoxyriboside, Rf 0.53-0.59. It was found that at least 1 mμg of vitamin B_{12} and 200 mμg of desoxyriboside are required to produce appreciable growth zones in this method.

A papain hydrolysate of sockeye salmon kidney and a 1 in 10 (pH 5.0) extract of lemon sole *(Parophyrs vetulus)* kidney heated at 100° C. for 30 minutes was chromatographed by this procedure. Marked growth zones resulted indicating that these preparations contained vitamin B_{12}, and probably the four desoxyribosides of guanine, adenine, thymine and cytosine. A sample of herring stickwater, containing 7.8 percent solids, gave a marked vitamin B_{12} zone and a slight

TABLE 1

Occurrence of vitamin B_{12}-active substances in certain fishery products

	(mμg of B_{12} per gram of dry material)
Fish Organs and Flesh	
Lingcod *(Ophiodon elongatus)* liver	430
Lingcod *(Ophiodon elongatus)* liver	880
Halibut *(Hippoglossus stenolepis)* liver	520
Dogfish *(Squalus suckleyi)* liver	96
Sockeye salmon *(Oncorhynchus nerka)* liver (Av. of 12 fish)	1,700
Sockeye salmon *(Oncorhynchus nerka)* kidney (Av. 12 fish)	18,000
Sockeye salmon *(Oncorhynchus nerka)* pyloric caeca (Av. of 12 fish)	3,100
Sockeye salmon *(Oncorhynchus nerka)* milt (Av. of 12 fish)	480
Sockeye salmon *(Oncorhynchus nerka)* spleen (Av. of 12 fish)	7,200
Sockeye salmon *(Oncorhynchus nerka)* stomach (Av. of 12 fish)	1,700
Sockeye salmon *(Oncorhynchus nerka)* eggs (Av. of 12 fish)	1,700
Dried fish liver meal (commercial product)	470
Spray dried fish liver extract (South Africa)	7,200
Lingcod muscle	180
Red cod *(Sebastodes sp.)* muscle	120
Mink food (ground halibut heads)	72
Lingcod viscera (as used for extraction of vitamin A containing oil)	85
Condensed Fish Solubles	
Tuna cooker juice (California)	460
Alaska herring *(Clupea pallassii)* (California)	670
Pacific sardine *(Sardinops caerulea)* (California)	460
Tuna waste (California)	270
Menhaden (California)	300
White muscled fish (California)	22
Commercial sample of unknown history	460
Commercial sample of unknown history	200
Commercial sample of unknown history	22
Dogfish (British Columbia)	78
Whale (Norwegian sample)	320
Sperm whale (British Columbia) (pH 6.8)	170
Sperm whale (British Columbia) (pH 4.5)	86
Stickwater Before Condensing	
Pink salmon offal *(Oncorhynchus gorbuscha)* (British Columbia)	530
Pink salmon offal *(Oncorhynchus gorbuscha)* (British Columbia)	620
Pink salmon and sockeye salmon offal (British Columbia)	520
Chum salmon offal (British Columbia)	530
Fish Meals	
Pink salmon offal (British Columbia)	190
Pink salmon offal (British Columbia)	190
Pink salmon and sole offal (British Columbia)	170
Chum salmon offal (British Columbia)	270
Mixed salmon offal (British Columbia)	360
Mixed salmon offal (British Columbia)	180
Whole Pacific herring (British Columbia)	1,300
Whole Pacific herring (British Columbia)	1,500
Herring meal (market sample of undetermined origin containing 50 percent protein)	310
Offal from white fleshed fish filleting operation	80
Meal prepared from fish waste by a solvent (trichlorethylene) extraction process	70
Meat meal (50 percent protein)	170
Little neck clams *(Paphia staminea)*	2,500
Tuna viscera digest	3,200
Wash water from vitamin A alkali extraction process of lingcod livers	210

zone corresponding to guanine desoxyriboside when 0.04 ml. was chromatographed by this method. Application of the KH_2PO_4 impregnated filter paper chromatography technique recently described by Woodruff and Foster *(24)* to this stickwater indicated that both vitamin B_{12} and B_{12a} were present. Quantitative determinations of vitamin B_{12} and B_{12a} in relation to total vitamin B_{12}-active substances in fish materials has not yet been attempted.

Microbiological Formation of Vitamin B_{12}-Active Substances in Stickwater

The stickwater which is pressed from cooked fish in the process of making fish meal forms a good potential culture medium for microorganisms since it contains a fair proportion of essential amino acids *(11)* and also vitamins of the B complex. Its composition is somewhat variable. The preparation used throughout the present work was a sample from reduction of whole herring and contained 7.8 percent solids, 0.95 percent ash and 0.85 percent chloride (NaCl). Samples were stored at —20° C. until required.

Eighty-five bacteria representing a large number of different genera, some obtained from culture collections and some isolated from soil samples, were grown for several successive transfers in vitamin B_{12}-free liquid medium *(17)*. Twenty-five of these formed exhibition zones when their growth medium was tested by cup-plate assay *(4)* on the same medium solidified with 2% agar. This medium was found to give good growth zones with solutions containing between 5 and 40 μg of thymine desoxyriboside per ml. When the above twenty-five cultures were re-tested using a cup-plate assay which is reputedly insensitive to desoxyribosides *(5)*, only fourteen gave positive exhibition growth zones indicative of their ability to form vitamin B_{12}-active substances. However, the amounts formed in this medium were not large, and did not exceed 50 mμg of vitamin B_{12} activity per ml. as judged by the above assay. Attempts to grow several of the spore-forming soil *Bacilli* which had been positive in the above experiment in undiluted stickwater were not very successful.

Further work showed that a *Serratia marcescens* culture which did not form vitamin B_{12}-active substances in the above vitamin B_{12}-free medium grew well in stickwater and increased the natural vitamin B_{12} content from about 120 mμg per ml. to an apparent 500 or 600 mμg per ml., as judged by cup-plate assay. This property was common to twenty different members of the genus *Serratia* which were examined. It was found that, when practically all measurable vitamin B_{12} activity was destroyed in stickwater by alkali treatment *(21)*, *Serratia marcescens* would grow on the neutralized product to form about 400 mμg. per ml. of vitamin B_{12}-active substances. When this product was again treated with alkali, neutralized, and assayed by cup-plate assay, the apparent vitamin B_{12} content had increased to about 1000 mμg. per ml. It would appear possible that at least some of the vitamin B_{12}-active substance which *Serratia* forms in stickwater are not vitamin B_{12} and may, perhaps, be similar in nature to the vitamin B_{12}-active substance formed by *Clostridium tetanomorphum (24)*. This suggestion is borne out by the fact that chromatographic separation of a *Serratia*-stickwater growth product on KH_2PO_4-impregnated filter paper *(24)* indicated that there was a large amount of some vitamin B_{12}-active substance present which did not appear to migrate from the point of origin.

It has been realized for some time that certain *Streptomyces* species, particularly *S. griseus (15, 19)*, form vitamin B_{12} and for this reason their ability to form this vitamin in stickwater is being investigated. Three cultures of *S. griseus* have been used, namely A.T.C.C. No. 10137, and two strains which had reputedly been employed to prepare industrial antiobiotic. The procedure adopted was to inoculate 25 ml.-portions of sterilized stickwater, with or without additives, with a small piece of the growth from a fresh nutrient agar culture of *S. griseus*, and to aerate the suspensions actively at 28° C. Samples for vitamin B_{12} assay *(5)* were withdrawn at intervals. Since serious frothing did not occur, the use of octadecanol as antifoam agent was discontinued after preliminary tests. Before assay the samples were usually diluted ten fold, adjusted to pH 5.0, and heated thirty minutes at 100° C. Except in one or two instances in which diluted stickwater was used, the exhibition zones formed on assay of these *Streptomyces*-fermented stickwaters had rather diffuse margins and therefore the results were not quantitatively very accurate.

Preliminary tests indicated that, although growth appeared to be more rapid in stickwater diluted with an equal volume of water, the amount of vitamin B_{12} formed was similar in the undiluted material. The addition of 1 percent glucose plus 0.4 percent calcium carbonate, with or without 0.1 percent sodium nitrate, appeared to have no effect on vitamin B_{12} formation. Under good conditions the amount of vitamin B_{12} formed by all three *S. griseus* strains reached a maximum after about two days' incubation, and thereafter the amount present decreased rapidly.

When the *S. griseus* stickwater fermentation liquors were heated at pH 5 and 0.01 ml. portions spotted on ordinary filter paper *(23)*, definite double zones with Rf values of roughly 0.03 and 0.06 were formed on the growth medium, indicative of vitamin B_{12}. In this concentration the growth zones characteristic of the various desoxyribosides did not develop. Further tests using KH_2PO_4-impregnated filter paper *(24)* indicated that vitamin B_{12a} and, possibly, vitamin B_{12}, were present in these fermentation products. It is hoped that further work will indicate the actual amounts of vitamin B_{12} and B_{12a} present in these fermentation suspensions.

Acknowledgments

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Effect of High-Voltage Cathode Rays on Amino Acids in Fish Muscle[a]

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Haddock fillets were irradiated with supervoltage cathode rays produced by a pressure-insulated, electrostatic Van de Graaff generator. Before and after irradiation, microbiological determinations were made of ten amino acids in the fillets, namely, arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and cystine. An organoleptic study of the fillets was also made by a taste panel of nine judges. The investigation indicates the possibility of sterilizing lean fish fillets by cathode ray irradiation without loss of amino acid content.

Introduction

A number of investigators have shown that ionizing radiations have a lethal effect on microorganisms *(12, 13, 14, 21, 28)*. The possibility of utilizing X-ray irradiation as a means of sterilizing foods was demonstrated by Proctor, Van de Graaff and Fram in 1943 *(19)*. Brasch and Huber showed later that they were able to sterilize a wide variety of medicines, therapeutics, and foodstuffs with penetrating electrons of ultrashort duration as emitted from the capacitron *(2, 3)*. Dunn, Campbell, Fram and Hutchins *(6)* found that X-rays and cathode rays destroyed bacteria, yeasts, and molds in massive concentrations in pure culture and when associated with liquid or solid substances such as milk, water, apple juice, ground spices, soil, and catgut sutures. They indicated the possibility of sterilizing fluids by a continuous process.

These investigations demonstrate that X-rays and cathode rays have potentialities as a means of sterilizing foods. If this is so, then information is essential on how they affect vitamins, enzymes, and other nutrients in foods. Extensive studies on the effects of X-rays and cathode rays on vitamins and enzymes have been carried out in the Food Technology Laboratories at the Massachusetts Institute of Technology for the past few years.

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Proctor and Goldblith *(10, 16, 17)* have studied the effects of ionizing radiations on niacin, riboflavin, ascorbic acid, and carotene. All these vitamins are affected adversely in dilute solutions. Other unpublished work in these Laboratories has shown that vitamins are relatively more radio-resistant in foods than in pure solutions of equivalent concentrations.

Investigations on the effects of X-rays and cathode rays on enzymes in foods have indicated that the enzymes are only slightly affected at the dosages necessary for sterilization *(6)*.

In addition to vitamins and enzymes, amino acids form another group of compounds of great importance. The authors are not aware of any published reports on the effects of cathode rays on amino acids in foods. Some research on solutions of amino acids has been done by other investigators. Stenström and Lohmann *(24)* studied the effect of Roentgen radiations on solutions of tyrosine and cystine. They found that tyrosine was decomposed by X-rays. No measurable change was noticed in cystine. Stein and Weiss *(23)* showed that X-rays destroy amino acids by deamination. Dale, Davies and Gilbert *(4)* have independently demonstrated deamination of amino acids and their derivatives by X-rays. Fricke *(9)* studied the denaturation of proteins by high frequency radiation. From the physiological point of view, the most important result of chemical changes produced in proteins by high frequency radiations is the loss or modification of their specific (catalytic, immunological, etc.) properties.

As the food technologist is interested in the nutritional and organoleptic properties of irradiated proteins, the present investigation was started on this basis. Haddock muscle was selected as the typical protein, because it is rich in all the "essential" amino acids. Moreover, Proctor, Nickerson and Goldblith *(18)* have shown recently that autolysis is not an important factor in the spoilage of haddock fillets.

Equipment

In this investigation only cathode rays were used. A pressure-insulated, electrostatic generator of the Van de Graaff type, operating at 3 million volts and capable of producing a continuous supply of monoenergetic electrons in a magnetically focused beam, was used for the production of the cathode rays *(20, 25, 26)*. The dosages of cathode rays are expressed in terms of roentgen-equivalents-physical (rep), as described by Evans *(8)*.

Experimental Methods

Irradiation: Three haddock were filleted. Two fillets were obtained from each fish. One fillet served as the control, and the other was irradiated. The fillets were flattened out before irradiation, and the maximum thickness of the fillets was 1 cm. This ensured thorough penetration of the entire mass by the cathode rays. The fillets, heat-sealed in polyethylene bags, were placed on a canvas belt and run under the cathode ray beam delivered by the Van de Graaff generator. The speed of the belt was predetermined, and the samples were exposed to cathode rays for a specified time at a given plate current. The dosage was regulated by the length of time of irradiation. The physical data on irradiation are shown in Table 1.

TABLE 1

Physical Data on Irradiation of Haddock Fillets

Average Dose	Plate Current	Exposure to Electron Beam	Operating Voltage
rep	*micro-amp*	*seconds*	*million volts*
900,000	60	8	2.8
2,700,000	120	16	2.8
5,700,000	200	24	2.8

The irradiation was done at three levels, namely, 900,000, 2,700,000, and 5,700,000 rep. The lower dosage of cathode ray energy was selected because this amount of energy has been found in the Food Technology Laboratories at the Massachusetts Institute of Technology to destroy all the viable microorganisms and spores in fish. The higher dosages were chosen with a view to ascertaining the extreme effects of irradiation.

Hydrolysis: Both the irradiated and the control samples were disintegrated into small pieces and then dried over a steam bath for 12 hours. The drying was finished by placing the material in an air oven at 90° C. for two hours. There was some exudate of tissue fluids in the polyethylene bags, which was carefully mixed into the flesh during disintegration. The dried samples were ground finely in a mortar and stored in glass bottles. The hydrolysis was carried out according to the method of Henderson and Snell *(11)*.

For alkaline hydrolysis, 1.5 gm. of each dried sample was weighed into a 2-oz., screw-cap, glass bottle, and 20 ml. of 5 N sodium hydroxide was added. The cap was screwed on tightly, and the hydrolysis was carried out by autoclaving for 15 hours at 15 p.s.i. The hydrolysates were adjusted to pH 6.8 and made to 100 ml. Alkaline hydrolysates were used for tryptophan and phenylalanine assays.

For acid hydrolysis, 1.0 gm. of the dried material was autoclaved with 40 ml. of 3 N hydrochloric acid for 5 hours at 15 p.s.i. The hydrolysates were adjusted to pH 6.8 and made to volume.

All hydrolysates were stored in the frozen state. The frozen samples were thawed immediately before analysis and refrozen after the aliquots were taken out.

Microbiological Determination of Amino Acids: In this study, determinations of ten amino acids were carried out, i.e., arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and cystine. The assay technique was essentially the same as that employed by Dunn, Shankman, Camien, Frankl and Rockland *(7)*, and the basal medium "D" developed by them was used. *Leuconostoc mesenteroides* P-60 was employed as the test organism for all the amino acids reported in this study. It was maintained in stab on a medium consisting of 1.5 percent agar, 1.0 percent glucose, and 1.0 percent Difco Bacto-yeast extract and was transferred to fresh medium every two weeks *(22)*.

Preparation of Inoculum: Prior to use in assay, the microorganisms were transferred to complete medium or the "Micro-Inoculum Broth" *(5)*. After incubation for 24 hours at 37° C., the cells were centrifuged and the broth was decanted. The centrifuged cells were washed twice with 0.85 percent saline under aseptic conditions and finally resuspended in 10 ml. of sterile

saline. One drop of this suspension was used for inoculating each tube. The washed cells were stored in the refrigerator overnight, if necessary. Otherwise absolutely fresh cells were used.

Setting Up the Culture Tubes: In each tube, 2 ml. of the basal medium was used. Each tube contained a final volume of 4 ml., distilled water being added to make up the volume. It was found that best mixing is ensured by first adding the distilled water to the tube, then the sample and finally the medium, half of it down the sides of the tube and the rest directly into the liquid. Each sample was assayed at five different levels of concentration of the test substance. Tubes prepared for assay were autoclaved for 10 minutes at 15 p.s.i., and incubation was carried on for 72 hours at 37° C. The acid produced in the tubes was titrated with standard base, bromthymol blue being used as the indicator.

Methods for Calculating Amino Acid Content: The results of the assays were calculated by the slope-ratio method *(27)*, except in those cases where the straight-line portion of the standard curve was not large enough. In such cases the "single-curve method" *(27)* was used.

Determination of Nitrogen: Nitrogen was determined by the Kjeldahl-Gunning-Arnold method *(1)*.

Organoleptic Tests on Irradiated Fish

The irradiated fillets were broiled for 15 minutes. The broiler pan was not greased, and no salt or lemon juice was used. The control and the irradiated fillets were broiled under identical conditions. The broiled fish were presented to a taste panel consisting of nine persons, four women and five men. Each person was given the control and the irradiated samples and was asked to answer the following questionnaire:

1. Do you like fish?
2. Which sample do you prefer?
3. Why do you prefer it?
4. Any other remarks?

Experimental Results

The results of this study will be presented in three sections, dealing with (1) the effects of cathode ray irradiation on the physical condition of the fish fillets, (2) the effects of such irradiation on the amino acids in the fish flesh, and (3) the organoleptic quality of the irradiated fish.

1. *Effects of Cathode Rays on Physical Condition of Haddock Fillets*

Trump and Van de Graaff *(26)* have shown that there is a 2° C. rise in temperature in a product for each million rep of cathode ray irradiation. By actual measurement, the rise in temperature of the fillet irradiated at 5.7 million rep was 13.7° C. (from 25.7° to 39.4° C.), which is in fair agreement with the finding of Trump and Van de Graaff. The other two fillets irradiated at 900,000 rep and 2.7 million rep did not show any perceptible rise in temperature. With an increase in the dosage of radiation, there was a progressive increase in the cooked and modified odor (Table 2). Some foam was noticed in the sample irradiated at the highest dosage; that fillet could not be removed from the polyethylene bag in one piece but was very fragile and was removed in small sections.

TABLE 2

Effect of Cathode Rays on Physical Condition of Haddock Fillets

Dosage of Irradiation	Appearance	Texture	Odor
rep			
900,000	No change	No change	Slightly cooked
2,700,000	Slight bleaching of surface; exudate of tissue fluid	Slightly crumbly	Marked cooked oxidized odor resembling ozone
5,700,000	Surface coagulation; large quantity of exudate of tissue fluid; surface bleached to absolute white	Very crumbly	Very marked cooked odor; strong smell of ozone

2. *Effect of Cathode Rays on Amino Acids*

The results of the amino acid determinations are shown in Table 3. In every case the content of amino acid has been expressed as parts of amino acid per 16 parts of nitrogen (approximately equivalent to the percentage of amino acids in the protein).

No significant destruction of any of the ten amino acids was observed in the present experiments, as seen from Table 3.

Recently Neilands, Sirny, Sohljell, Strong and Elvehjem *(15)* have reported a study on the reliability of microbiological methods for amino acid determinations. They examined the inter-laboratory agreement in the results obtained in two different laboratories with 41 common samples assayed for ten amino acids. To express the degree of variation among these results, they

TABLE 3

Effect of Cathode Rays on Amino Acids in Haddock Fillets

Amino Acid and Dosage of Cathode Rays	Amino Acid Content* Control	Amino Acid Content* Irrad.	Change in Amino Acid Loss %	Change in Amino Acid Gain %
Phenylalanine				
900,000 rep	3.63	3.73	0.00	2.75
2,700,000 rep	3.86	3.77	2.33	0.00
5,700,000 rep	3.93	3.69	6.10	0.00
Tryptophan				
900,000 rep	1.06	1.02	3.77	0.00
2,700,000 rep	1.02	0.92	9.80	0.00
5,700,000 rep	1.16	1.08	6.92	0.00
Methionine				
900,000 rep	2.86	2.84	0.70	0.00
2,700,000 rep	2.85	2.69	5.61	0.00
5,700,000 rep	2.99	2.85	4.68	0.00
Cystine				
900,000 rep	1.02	0.99	2.94	0.00
2,700,000 rep	1.01	0.94	6.93	0.00
5,700,000 rep	1.04	1.04	0.00	0.00
Valine				
900,000 rep	6.42	6.55	0.00	2.03
2,700,000 rep	6.32	6.24	1.27	0.00
5,700,000 rep	6.29	6.69	0.00	6.36
Leucine				
900,000 rep	7.89	7.74	1.90	0.00
2,700,000 rep	7.92	7.44	6.06	0.00
5,700,000 rep	8.03	8.25	0.00	2.74
Histidine				
900,000 rep	1.98	1.97	0.50	0.00
2,700,000 rep	2.02	1.85	8.41	0.00
5,700,000 rep	1.85	2.00	0.00	8.11
Arginine				
900,000 rep	5.61	5.48	2.32	0.00
2,700,000 rep	5.63	5.53	1.78	0.00
5,700,000 rep	5.34	5.56	0.00	4.12
Lysine				
900,000 rep	10.47	9.80	6.40	0.00
2,700,000 rep	9.52	9.52	0.00	0.00
5,700,000 rep	9.70	9.29	4.23	0.00
Threonine				
900,000 rep	4.37	4.41	0.00	0.91
2,700,000 rep	4.13	4.29	0.00	3.87
5,700,000 rep	4.87	4.58	5.95	0.00

*Amino acid content expressed as parts of amino acid per 16 parts of nitrogen.

averaged all values for a given amino acid and expressed the standard deviation between the two laboratories as a percentage of this mean. The coefficients of variation so calculated ranged from 5.2 percent for histidine to 17.5 percent for tryptophan, and the average coefficient of variation for all amino acids was 8.9 percent.

The same authors reported also that the coefficient of variation among replicate determinations made with a given test organism in their laboratory ranged from 3.5 percent for leucine assayed with *L. delbrueckii* to 12.0 percent for methionine assayed with *L. fermenti*, and that the average coefficient for all amino acids and all test organisms was 8.0 percent.

In the light of the study reported by Neilands et al., there is justification in concluding from the present investigation that the cathode ray irradiation had no adverse effect on the amino acids determined in the haddock fillets.

In general, lysine, methionine, and tryptophan are the three essential amino acids considered most important in evaluating the nutritive quality of a protein *(15)*. These three amino acids, in the present study, showed maximum destructions of 6.4, 5.6, and 9.8 percent, respectively, which are within the range of duplication limits of microbiological determinations. On the basis of these results, it may be stated that haddock fillets can be sterilized adequately by cathode rays, without adverse effect on the ten amino acids in fish muscle that were considered in this investigation.

3. *Organoleptic Quality of Irradiated Haddock Fillets*

The results of the taste tests are shown in Table 4. Because of the limited number of judges on the taste panel, no valid conclusions can be drawn from these tests. However, the results suggest that there may be some persons who will be unable to detect any appreciable change in the flavor of broiled haddock that has been exposed to a dosage of cathode ray irradiation sufficient for sterilization of the fish.

TABLE 4

Results of Organoleptic Tests

Dosage	Judges Preferring		Judges Unable to Detect Any Difference
	Control	Irrad.	
rep			
900,000	3	1	5
2,700,000	3	2	4
5,700,000	6	1	2

Summary

Fish fillets (haddock) were irradiated by cathode rays at three levels, namely, 900,000, 2,700,000, and 5,700,000 rep.

Before and after irradiation, assays were made for ten amino acids, namely, arginine, histidine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine, and cystine. No significant destruction of any one of these amino acids was noticed as the result of such irradiation.

Irradiated and non-irradiated haddock fillets were examined by a small taste panel. The fillets subjected to sterilizing dosages of cathode rays and later broiled were as acceptable to some judges as the broiled, non-irradiated fillets.

The feasibility of sterilizing haddock fillets on a belt moving under a cathode ray beam has been indicated. None of the amino acids investigated are destroyed in this process of sterilization by irradiation.

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Confectionery Sweeteners[a]

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Review Paper

A comparison is presented showing the composition of the major types of sweetening ingredients represented by sucrose and sucrose syrups; invert sugar and invert sugar-sucrose syrups; corn syrups—both regular and high dextrose equivalent; and dextrose.

Some of the more significant physical and chemical properties are discussed, namely, solubility, crystallization, inversion and stability to color development. The value of technological control on raw materials, processing and finished products is stressed.

Emphasis is placed upon the importance of having a thorough knowledge and understanding of the composition, properties and reactions of sweeteners in order that conditions leading to their most efficient use be established, problems solved, and uniform, high quality merchandise be produced.

This discussion is concerned with confectionery sweeteners—the backbone of the confectionery industry. The material is not new but should directly concern every progressive confectioner. During the past few decades confectionery manufacturing has grown into a major and important industry. However, most of the present state of knowledge or "art" is empirical and has been obtained from innumerable small improvements by individual confectioners and suppliers and not by concerted action through the industry. Modern progress depends more and more upon the application of sound technological knowledge and skills both within the confectioner's own organization and from outside sources. For the confectioner to obtain the most efficient use of sweeteners, with the viewpoint of producing uniform, high quality merchandise with a minimum of processing or product problems, a thorough knowledge and understanding of the composition, properties and reactions of these materials is a requisite.

In establishing quality specifications or grades for the sweeteners used by the confectioner the suppliers endeavor to furnish materials which fulfill the needs and demands of the industry. These needs and demands, in almost all cases, are dependent upon the properties desired in the product, by the equipment available to manufacture the product, the reactions involved in the process and the product objectives, i.e., the type of market, price, shelf life, and so forth. The manufacturers of sweeteners make every effort to provide scientifically standardized, uniform quality products. They also make readily available all of the necessary and pertinent information on these products which is important to the industry.

Confectioners should purchase and use sweeteners not because of price alone or hypothetical or fanciful properties but because of known and proven desirable characteristics inherent in the raw materials. In other words, confectioners should use the various sweeteners to do a particular job or series of jobs. For example, sucrose or sucrose products should be used because of their value as a sweetener, their ready solubility, their function as a "drier" to shorten the body of the confection through crystallization and their unique property of affording "doctor" by the formation of invert sugar. Invert sugar, which is derived from sucrose, possesses the very desirable physical property of controlling the crystallization of concentrated sugar solutions and maintaining freshness in the finished product by reason of its hygroscopicity. Corn syrup is used to afford "body" or viscosity, as a "doctor" to prevent sucrose crystallization, to reduce sweetness and, to a degree, afford whipping properties. Dextrose may be used to modify sweetness and change solubility characteristics in the confection.

In order for the confectioner to understand better the proper use of sweeteners to do a particular job it is important, first of all, that the composition of these products be known since many of their properties are dependent upon the composition. Obviously, it is not feasible to discuss all of the grades, types and variations of these ingredients nor is it possible to include general or specific information on many of the newer products resulting from such recently developed processes such as ion-exchange. Inasmuch as products derived by ion-exchange or other improved and new processes must still be considered to be in an experimental stage of development they are not included in the following discussion. Specific information on these products should be obtained from the companies specializing in their manufacture.

[a] Presented before the Tenth Annual I.F.T. Convention, Chicago, Illinois, May 23, 1950.

Let us first consider the composition of sucrose (sugar) products. Table 1 shows the typical compositions of two representative grades of granulated sugar, two grades of uninverted sugar syrups and two representative types of invert sugar syrups.

In the case of the granulated sugars the two major classes are shown, the larger grain "first run liquor" sugars illustrated by Medium Granulated and the finer grain sugars as shown by Fine Granulated. All of the grades of the larger grain sugars such as Coarse, Standard, Medium and Sanding have essentially the same composition and differ only in their particle sizes. These grades of sugar, in some cases, are known as brilliant sugars since they possess a clear, sparkling luster produced by drying under special conditions to minimize surface scratching and dulling. The finer grain sugar grades such as Fine, Extra Fine, Fruit or Berry and Coating are essentially of the same composition and differ mainly in their particle sizes. This wide variety of sugar grades are offered by the refiners since individual confectioners vary in their opinions as to the sugar grade specifications they consider necessary for a given purpose.

It is important to note that properly refined granulated sugar grades are of almost equivalent purity and vary but slightly in the percentage of non-sugar components. By purity we mean the almost complete absence of ash and organic matter. Table 1 shows that the difference in the order of magnitude of the ash, for example, between the Medium and Fine Granulated grades of sugar is only a matter of about .005 percent. This exceptional degree of purity is obtained by refiners through the highly efficient and effective process of crystallization and recrystallization, abetted by special purification procedures utilized while the sugar is in solution.

We have not shown the composition of "soft" sugars, which are also commonly known as brown or yellow sugars, since they are of limited usage to the confectioner. However, a short description of these may be of general interest. They are a group of intermediate cane sugar products classified according to a series of numbered arbitrary color standards. They consist essentially of sucrose crystals surrounded by relatively thick films of syrup. These sugars are of primary importance to the confectioner for their cane-molasses flavor and, to some extent, for their color. They contain from 1-4 percent ash, from 2-6 percent invert sugar and approximately 4 percent moisture. The flavor of soft sugars may vary slightly at times which is more or less beyond the control of the refiner partly due to the fluctuating composition of the non-sugar components in the raw sugar from which they are refined and partly to the particular refining processes used.

The uninverted sugar syrups shown in Table 1 are those commonly known to the various food industries using them and are titled with their general, common usage and accepted names, i.e., No. 1 and No. 2 Sugar Syrups. The main difference between these two grades lies in the very light straw color and slightly higher percentage of non-sugar components of the No. 2 syrup as compared with the clear, water-white No. 1 syrup.

These sugar syrups, dependent upon the quality of the product desired, can be and are being used satisfactorily, by proper balancing of formulae, for all general confectionery products wherein a solution of sugar in water is required. Possible exceptions may be the manufacture of pure white fondants and crystal clear, hard candies that are processed with the equipment and procedures usually employed by the confectionery industry.

Table 1 shows that approximately 33 percent water is present in these uninverted sucrose syrups. If this percentage is greater than that normally employed in a process it must be removed by slightly extending the boiling or cooking time or by adjusting the formula if other water containing ingredients are used. The expedient of adding dry sugar (known as "stuffing" the batch) to increase the sugar concentration equivalent to that normally used also can be employed.

The partially and totally inverted syrups are shown simply to illustrate the composition of this wide category of sweeteners. These syrups may range from as low as 10 percent or less invert sugar to as high as 95 percent invert sugar; 100 percent inversion is seldom, if ever, obtained in commercial manufacturing processes. These products are obtainable at considerably higher densities than the uninverted type syrups.

It should be noted that the ash percentages shown are those represented by the so-called Manufacturers Grades or the light straw colored syrups. The special, water white grades may contain ash percentages as low as 0.02 percent depending upon the manufacturing process used. The pH, of course, may be adjusted at the refinery to any value desired by the confectioner,

TABLE 1

Typical Composition of Sucrose Products *

Types	Granulated Sugar		Uninverted Syrups				Inverted Syrups			
Grades Represented by	Medium Granulated	Fine Granulated	No. 1 Syrups		No. 2 Syrups		Partially Inverted		Totally Inverted	
	Percent	*Percent*	*Percent*		*Percent*		*Percent*		*Percent*	
			Wet Basis	*Dry Basis*	*Wet Basis*	*Dry Basis*	*Wet Basis*	*Dry Basis*	*Wet Basis*	*Dry Basis*
Moisture	0.02	0.04	33	....	33	....	27	....	24	....
Total Solids	99.98	99.96	67	100	67	100	73	100	76	100
Sucrose	99.95	99.90	66.6	99.5	66.3	99.0	42.5	58	3.5	4.5
Invert Sugar	0.01	0.03	0.1	0.15	0.4	0.6	30	41	72	94.5
Ash	0.005	0.01	0.05	0.075	0.08	0.12	0.1	0.14	0.12	0.16
pH	....	....	6.5-7.0		6.5-7.0		5		5	
Relative Solution Color	Clear Water White	Essentially Water White	Clear Water White		Clear Light Straw		Clear Water White to Light Straw		Clear Water White to Light Straw	

* Based upon Eastern Cane Refiners' Products.

however, it is usually kept around 5 primarily to stabilize the color of the syrup.

A very good chart showing the safe densities for the storage of invert syrups in the possible presence of seed crystals has been published by Junk, Nelson and Sherrill *(6)*.

Table 2 illustrates the approximate composition of the various types of corn syrups commonly used by the confectioner *(7, 2)*. The corn syrup represented by the name "Regular" is the syrup used in the largest quantity by the confectionery industry. The Medium conversion corn syrup is used primarily for special application in marshmallow production. The abbreviation "D. E." shown refers to the dextrose equivalent, which means the total amount of reducing substances expressed as dextrose on a dry basis and this value is lower than the actual sum of the total sugars present. It is important to bear in mind that the Baumé degrees shown are those determined at 37.8° C. (100° F.) and that all commercial corn syrup densities, expressed in degree Baumé, are referred to 37.8° C. (100° F.).

The basis for choosing one of the corn syrup types shown in Table 2 rests on the premise of whether the confectioner wants to obtain as much relative sweetness as possible or whether the syrup is to be used primarily for its viscosity or "body" effect or crystallization control functions. An examination of the compositions show that the dextrine content of the syrups range from the highest in the regular type to the lowest in the high conversion (dual acid-enzyme) types while the reverse is true of the sugar (maltose and dextrose) content. The percentage of the dextrines present determines to a large degree the "body" or viscosity of the syrup while the sweetness factor is primarily due to the amount of the sugars, dextrose and maltose, present.

Another sweetener available to the confectioner is dextrose and there are two forms manufactured. The approximate composition of these two forms of dextrose are shown in Table 3. The most commonly used is the hydrated form which is a white, crystalline material containing one molecule of water of crystallization. The moisture content shown corresponds very closely to this molecule of water of crystallization although in commercial practice somewhat less water may be present, i.e., the moisture content will range from 8-9 percent. The anhydrous form has the water of crystallization removed by proper processing and is a white, crystalline, relatively hygroscopic material.

TABLE 3

Typical Composition of Commercial Dextrose

Type	Anhydrous	Hydrated
	Percent	*Percent*
Moisture	0.2	9.0
Total Solids	99.8	91.0
Dextrose	99.78	90.98
Ash	0.02	0.02

Since the confectioner is concerned with the practical utilization of sweeteners as determined by their physical properties such as solubility, crystallization, inversion, stability to color development, sweetness and viscosity it is important that he understand these properties thoroughly as related to his products.

While some of these properties are common to the various sweeteners, differing only as to degree, others may be specific for a particular sweetener. We know, for instance, that the ability of invert sugar and corn syrup to retain moisture is a property common to each. Corn syrup also has a common property that is not present, however, to such a marked degree in invert sugar, sucrose and dextrose solutions, i.e., its extremely high viscosity. Sucrose, to illustrate a specific property, hydrolyzes to form invert sugar and this property is not common to corn syrup or dextrose. It should be recognized furthermore that, in most instances, the reactions and properties of the various sweeteners may be affected or modified by the other materials with which they are combined and the conditions attendant upon the processes employed.

Now, let us consider a few of the basic and useful properties of the individual sweeteners. *Sugar (Sucrose):* Sugar has the highest rate of solution of commercially available solid sweeteners and the smaller the crystal size the more rapid the solution under conditions of equivalent agitation *(10)*. Sugar, fortunately, forms no hydrate under ordinary conditions and, therefore, goes directly into solution without difficulty. Moreover, the size of the crystal does not influence the amount of sugar that can be dissolved, i.e., one pound of water at room temperature (about 20.0° C. [68° F.]) will dissolve two pounds of any grade or crystal size of sugar.

Sugar has the ability to form highly supersaturated solutions and these solutions possess the very desirable physical property of withstanding supercooling. Thus, a saturated sugar solution can remain liquid at temperatures considerably lower than those where crystallization normally occurs.

TABLE 2

Approximate Compositions of Corn Syrups

TYPES	Regular 43° Baumé—42 D.E.		Medium Conversion 43° Baumé—52 D.E.		High Conversion (dual acid-enzyme) 43° Baumé—63 D.E.		High Conversion (straight acid) 43° Baumé—60 D.E.	
	Percent		*Percent*		*Percent*		*Percent*	
	Wet Basis	*Dry Basis*	*Wet Basis*	*Dry Basis*	*Wet Basis*	*Dry Basis*	*Wet Basis*	*Dry Basis*
Moisture	19.7	....	19.0	....	18.5	....	18.5	....
Total Solids	80.3	100	81.0	100	81.5	100	81.5	100
Dextrose	18	22	26	32	30.5	37.5	33	40.5
Maltose	17	21	21	26	28	34	23	28
Higher Sugars	16	20	11	13.5	13	16	6.5	8
Dextrines	30	37	23	28	10	12	19	23
Ash	0.3	0.37	0.3	0.37	0.3	0.37	0.3	0.37
pH	4.7-5.0		4.7-5.0		4.7-5.0		4.7-5.0	
Approximate Relative Viscosity @ 100° F.	160 poises		104 poises		60 poises		80 poises	

One of the most important of the chemical reactions of sugar is its ability to hydrolyze or "invert" readily producing invert sugar. This reaction can be accomplished by relatively simple treatment with an appropriate acid or enzyme under controlled conditions.

While pure sucrose, per se, in solution is stable to color development it has been shown that it is acidic in its properties and this property is greatly enhanced at high temperatures resulting in auto-inversion *(1)*. The color that is developed in sucrose solutions has been demonstrated to be derived from the presence of invert sugar and is due mainly to the formation of polymers from 5-(hydroxymethyl) furfural produced by the decomposition of the invert sugar, particularly its levulose fraction *(11)*. The color developed in acidic solution is a function of the pH and color cannot be formed unless inversion has first taken place.

Since many minerals have acidic properties and inasmuch as there is present in all commercial, highly refined grades of sugar minute percentages of minerals (ash) inversion may take place, particularly at high temperatures. Thus, the percentage of ash and possibly other non-sugar components may be of some significance when making confections with the present day equipment and processes available to the confectioner.

While the possible significance of the ash and other non-sugar components in sugar should not be overlooked for certain high temperature processed confections it is important to point out that the ash contents of other ingredients are also of considerable import since these ash contents are, in general, present in larger amounts than in sugar and their potential action must be taken into consideration.

Finally, highly concentrated supercooled solutions of sugar at temperatures such as used for fondant beating have a relatively high viscosity and therefore contribute to the control of the crystal size during crystallization *(9)*.

Invert Sugar Syrups: These syrups possess the well known physical properties of being hygroscopic and retarding the crystallization of concentrated sugar solutions. Invest syrups discolor under heat *(6)* and must be used, either when formed in or added to the batch, in such manner as to minimize excessive color formation.

Corn Syrups: Corn syrups possess the physical property of retarding and controlling the crystallization of concentrated sugar solutions. This is due mainly to the dextrines present and thus the low conversion type syrups are more effective than the high conversion types *(7)*. Corn syrups afford viscosity and "body" to the confection also because of the dextrines present. These dextrines behave, to some extent, as protective colloids entrapping air and thus contributing to whipping power *(5)*. Interestingly, the higher conversion syrups are better suited for whipped products because of their greater fluidity *(2)*. Corn syrups, particularly the high dextrine or regular types, are relatively less sweet than sucrose or invert sugar. Corn syrups also may discolor at elevated temperatures because they contain reducing sugars which may decompose and therefore, care should be taken in their use under these conditions.

Dextrose: This sweetener possesses the ability to change solubility characteristics and modify the relative sweetness of confections. Figure 1 shows that dextrose has a lower solubility, at temperatures below 60° C. (140° F.), than either sucrose or invert sugar and thus has a higher degree of supersaturation at equivalent concentrations. Dextrose also tends to crystallize more slowly than sucrose and the solutions at the same concentrations are less viscous. Dextrose crystals are soft and do not take a polish well (i.e., if used in panning operations) *(7)*. Dextrose, while decreasing the solubility of sucrose due to the well known "salting-out" effect, allows the total sugar concentration to be increased over the solubility limits for either dextrose or sucrose. For example, at 30° C. (86.0° F.) sucrose dissolves to form a saturated solution of about 68 percent solids while dextrose dissolves to the extent of only 54.6 percent. However, the most soluble mixture solution of dextrose and sucrose will contain about 47 percent sucrose and 27 percent dextrose or a total solids content of approximately 74 percent *(4)*. Dextrose is also heat sensitive and tends to discolor at elevated temperatures *(11)*.

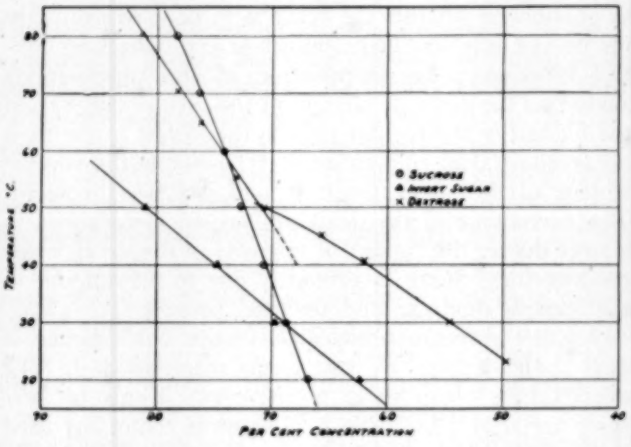

FIG. 1. Solubilities of sucrose, dextrose and invert sugar in water.

Let us consider briefly the practical application of some of these properties to a basic confectionery process and product, for instance, clear hard candy. Hard candy is essentially a highly supersaturated, supercooled solution of sucrose containing one percent or less of moisture. This solution, which may be likened to a glass, is usually prevented from crystallizing by the addition of invert sugar (either added per se or formed in the batch) or corn syrup or both, depending upon the properties desired. Thus, if the hard candy is to be relatively slow dissolving a larger proportion of corn syrup would be used; if a quicker dissolving product is desired smaller amounts of corn syrup are desirable, depending upon the requirements. Invert sugar while modifying other physical properties does not affect the sweetness significantly. However, if too much corn syrup and/or invert sugar is added to or formed in the batch, the confection will tend to be sticky and will absorb moisture readily causing further stickiness and even solution *(10)*.

Clear hard candies are not usually made with dextrose because it modifies the viscosity of the cooked and cooled batch to such a degree that unsatisfactory results may be obtained. Graining may also occur since dextrose has a lower solubility and does not form supersaturated, supercooled solutions so readily.

High conversion corn syrups, if used, may result in stickiness due to their higher hygroscopicity. These syrups also are not as resistant to color development at high temperatures as the regular or low conversion type and therefore the low conversion syrups are preferred for hard candy work *(8)*.

Since invert sugar and corn syrups are somewhat prone to discoloration at high temperatures excessive color formation may be minimized by adding these ingredients as late in the process as feasible or if "doctoring" is accomplished by the addition of inverting agents in the batch, delaying the addition of these materials until as late as possible. Lower temperature cooking, that is under vacuum, also helps minimize excessive color formation.

The term sweetness has been mentioned several times and a word of caution as to its significance is desirable. Relative sweetness values have been variously assigned to the many sweetening agents and have been determined usually on solutions of the pure materials at given concentrations under specific conditions *(3)*. These figures have, in general, almost no practical value to the confectioner since the conditions under which they were determined are not the same as those the confectioner uses when testing his products or even the raw materials themselves. Thus, many overly positive and misleading statements have been made about sweetness in the industry. While the statements may have been true, since sweetness is a matter of opinion, unfortunately the qualifying conditions are not usually given. Therefore, in order to evaluate the sweetness between any series of products they must be prepared under identical conditions and the measurements made under as near the conditions and circumstances of consumption as can be simulated. It is to no avail to consider sweetness an absolute figure and use it as such without careful tests on the products themselves since it can be markedly modified by the conditions under which it is tested, the composition of products being tested and the well known "human element" factor.

From the foregoing the confectioner can readily understand that a thorough knowledge and understanding of the composition, properties and reactions of the sweeteners they employ is of considerable import. The value of chemical and technological control on the raw materials, materials in process, and the finished product is an obvious concomitant. For example, the confectioner may wish to control the crystallization, modify the sweetness, decrease the hygroscopicity, increase the viscosity or body, or minimize color development in a product. The technologist or chemist using his training and experience and aided by the ready availability of basic technical data will tackle these problems in such a manner as to uncover the basic factors that influence any or all of these properties under a variety of conditions. He then extracts from this background those facts and data, which may not even be related to confectionery manufacture, he thinks are pertinent to the problem. This information is then applied to the problem and while it may be necessary, at times, that some compromise be made in order to solve the problem, in general, definite help can be secured most of the time.

Thus, if the confectioner is assured, through technological control, that his raw materials are what they should be, that his processes are under exact control and are thoroughly understood, he will be able to produce uniform, high quality merchandise at all times. He should be aware that there is a great deal of useful fundamental and practical information which can be applied to his problems, available in the literature, through the suppliers who are specialists in their fields and through the talents of the technologist. However, the problems must be stated in such a way as to allow the information to be used. Here the chemist and technologist working in your laboratory and in the laboratories of your suppliers can be of inestimable help.

It is the responsibility of the confectioner to fortify his own organization with technical skill, keep abreast of the new ideas and processes being developed and take full advantage of the technical service that is offered by the suppliers through trained technologists to gain uniform quality and improve quality control for his confections.

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Studies on Meat II[a, b]

Observations on Relation of Biochemical Factors to Changes in Tenderness

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Of the biochemical factors studied alkali insoluble protein and muscle plasma as represented by muscle hemoglobin (myoglobin) seem most closely correlated with changes in tenderness of beef.

In the first paper of this series Husaini, Deatherage Kunkle and Draudt *(4)* reported a relation between alkali insoluble protein and the tenderness of meat after aging for 14 days at 3.5° C. (38° F.). Their test samples were from market cattle of wide variation in market grades. In this paper a study is reported concerning some of the factors relating to changes in tenderness.

At The Ohio Agricultural Experiment Station a study was carried out on the relative efficiency of identical feeding management on feeder steers,—20 choice Herefords, 20 medium Shorthorns and 20 common Holsteins. Upon completion of that study 10 of the Herefords and 10 of the Holsteins were made available for this study. This selection was made to represent possible extremes among the cattle. The steers were purchased in November 1948 at the age of approximately 1½ years. They were wintered and put to pasture in the spring, and 90 days prior to slaughter were put on grain plus pasture. These animals were slaughtered at the Meats Laboratory of the Animal Husbandry Department. These steers are numbers 21 to 41 inclusive, excepting 37. Detailed studies on cost, dressing percentage, yield of edible portion, etc. of these steers will be reported in another paper.

From the herds of the Animal Husbandry Department four additional steers were included in this study on the same basis as those from the Experiment Station. These steers were all yearlings and full fed and suckled since birth. These animals are according to serial number, breed, and carcass grade: 37, Shorthorn, High Choice; 42, Hereford, Choice; 43, Shorthorn, High Good; 44, Angus, High Choice.

These two groups of steers were slaughtered and studied at the rate of four per week.

For studies on tenderness and changes in tenderness, one short loin from each steer was excised at 3 days *post mortem* and tested for tenderness and at the same time various biochemical constituents were determined. Most of these determinations were repeated at 15 days *post mortem*. Aging was done at 3.5° C. (38° F.).

This study was designed with the objective of trying to get some biochemical bases for tenderness of meat at 3 days and at 15 days *post mortem*. Husaini, et al. *(4)* have shown that the amount of alkali-insoluble protein was related to tenderness of meat at two weeks *post mortem*. The total alkaali insoluble protein is a measure of connective tissue. Furthermore some of the findings of Husaini, et al. support the suggestion of Deatherage and Harsham *(3)* that at least two major factors are related to tenderness in meat, namely, connective tissue and muscle plasma. Therefore some measure of active muscle plasma was sought. It was decided to run nitrogen partition as reported in the previous paper at 3 and 15 days as one possibility of detecting autolytic changes in the plasma. One other possibility was that muscle hemoglobin might be an index to the amount of muscle plasma or to the enzyme activity in the plasma. To test these hypotheses tenderness, alkali-insoluble protein, nitrogen partition, muscle hemoglobin, moisture, intramuscular fat and pH were determined at 3 days *post mortem*. At 15 days *post mortem* tenderness, alkali-insoluble protein, and nitrogen partition were run. The other factors determined at the three day level were found to remain unchanged at the 15 day level.

Methods

Most of the methods used in this study were the same as reported by Husaini, et al. *(4)*.

Sampling. Since in this study tenderness and some biochemical factors were determined twice, the excised short loins were sampled at the 3 day level, as reported before, and the remainder of the loin was kept until 15 days *post mortem*. The exposed anterior face of the loin was trimmed ¼ inch and then one 1½-2 inch section was cut for laboratory analyses and four 1-inch steaks were cut for tenderness determinations.

Alkali-insoluble protein. After this investigation was well under way it was apparent that results on alkali-insoluble protein were at variance with earlier results. The values were somewhat higher than expected although the reproducibility of triplicate determinations was satisfactory. Since the killing schedule had to be met and since the available time for laboratory analyses did not permit investigation of the difficulty it was decided to proceed through the entire series of animals without changing the technique as far as these carcasses were concerned.

Aside from the variation in animals, two sources for the poor agreement with previous work were considered. First, in view of some interference of fat in the determination, it was expected that low room temperatures 23° C. (73.5° F.) caused solidification of fat which prevented complete digestion by the alkali. There was also the temperature factor itself, since earlier work had been done during the summer when room temperature was 33 ± 2° C. (91.5 ± 3.5° F.). Second, there might be sampling differences. To test these hypotheses four carcasses were purchased, aged 14 days and ten-

[a] Presented before the Tenth Annual I. F. T. Convention, Chicago, Illinois, May 22, 1950.

[b] *Studies on Meat I. The Biochemistry of Beef as Related to Tenderness* was published in the August issue.

derness and alkali-insoluble protein were determined with digestions at 23° C. (73.5° F.) and at 35° C. (95° F.) and at different positions along the loin. From these results it was apparent that one difficulty was the temperature of digestion. For best results the higher digestion temperature is recommended rather than room temperature, whatever that may be. These animals are referred to as numbers 44 to 48 inclusive. Animal numbers 46 and 47 were 2½ years old steers grading High Good; number 45 was 2½ year old steer, Commercial; and number 48 was a 5 year old cow.

One other factor considered as a source of disagreement between the previous work and that reported here involves the tendonous intersection which often invades well into the center of the longissimus dorsi. It was discovered after completion of these studies that the assistant grinding the meat sample in the first study always discarded this fascia which remained on the grinder blade. Apparently this was the proper procedure for the tasters usually discounted this fascia in their scoring, as apparently it does not contribute to the toughness of the muscle itself. (In tenderness testing the steaks are cut so that only two out of the six judges have this tissue in their samples.) In the second study the assistant very conscientiously included this gristly membrane in the meat sample by altering the grinding procedure. It appears that this should not be done, and this point is under investigation.

Muscle hemoglobin. There are two pigments in meat, viz: blood hemoglobin and muscle hemoglobin, sometimes called myoglobin. In this work there was not sufficient time to accurately determine each by combining the procedures of Watson *(9)* and Bowen *(1)*. It was necessary to devise a simple procedure which would serve the purpose of this study and could be determined rapidly with perhaps some sacrifice of accuracy. Spectrophotometric measurement offered the most promise. Bowen *(1)*, Shenk, Hall and King *(6)* and Theorell *(8)* have made studies of the absorption spectra of blood and muscle hemoglobins and have shown a common absorption peak in the region of 542 mμ. Furthermore, Shenk, et al. *(6)* found that usually of the hemoglobins present in meat only 10% or less was blood hemoglobin. Therefore the measurement of optical density of aqueous extracts at 542 mμ would be an index of muscle hemoglobin within an error of 5 to 10%. Therefore the following procedure was adopted:

Twenty-five grams of meat was blended with 125 ml. water in a Waring blendor for 3 minutes. Then 0.15 grams of powdered sodium carbonate was added and the mixture was shaken and filtered on a quantitative filter paper. The first few drops were discarded and the filtrate usually was quite clear. If the filtrate was not clear, filtration was repeated. If this failed, a little calcium chloride was added to flocculate the colloidal material. The optical density of the clear filtrate was determined at 542 mμ using a Beckman D. U. quartz spectrophotometer. Using the absorption factor of 1.40 mg. per ml. for muscle oxy-hemoglobin as reported by Shenk, et al. *(6)*, and allowing for dilution, multiplication of the optical density by a factor of 0.84 gives the percent total hemoglobin in the meat. Of this hemoglobin 90-95% is muscle hemoglobin.

Results and Discussion

Partition of the nitrogenous constituents into zinc hydroxide-soluble, trichloracetic acid-soluble, and total nitrogen was determined as in the previous investigation on the first eight animals under investigation (number 21-28 inclusive) at 3 days and 15 days *post mortem*. There was only a slight increase in the trichloracetic acid soluble nitrogen from an average of 0.442% to 0.454% and there was no change in the nitrogen soluble in zinc hydroxide. Inasmuch as the carcasses showed increases in tenderness it was concluded that the protein changes responsible for increased tenderness on aging were too subtle to be determined by this technique. Consequently further work in this direction was discontinued.

Table 1 shows the intramuscular fat, moisture, total nitrogen and pH of the experimental animals excluding numbers 45-48. The data are separated as to 2½ year old steers from The Ohio Agricultural Experiment Station and the yearling steers from the Animal Husbandry Department herds. It is well to note the relative uniformity of these constituents in the Station cattle indicating some degree of similarity probably arising from identical management for one year prior to slaughter. The only major differences between these cattle and the yearling steers are in the values for intramuscular fat and their reflection in moisture and total nitrogen values.

TABLE 1

Fat, Moisture and Total Nitrogen Contents and pH of Experimental Meat Samples

Animal No.	Fat %	Moisture %	Total N wet basis %	pH (3 day level)
21	3.24	77.2	3.48	5.42
22	2.37	77.0	3.49	5.42
23	2.92	77.2	3.54	5.43
24	2.23	76.9	3.52	5.43
25	1.45	75.0	3.69	5.51
26	2.45	75.4	3.52	5.46
27	1.34	75.0	3.70	5.41
28	2.26	75.2	3.55	5.40
29	2.86	73.9	3.57	5.40
30	1.57	74.6	3.56	5.50
31	2.29	74.5	3.49	5.41
32	1.86	75.0	3.49	5.52
33	2.16	75.3	3.52	5.49
34	1.53	75.5	3.65	5.52
35	2.83	73.8	3.63	5.50
36	2.72	74.7	3.62	5.57
38	1.35	74.6	3.70	5.30
39	2.75	73.4	3.68	5.37
40	2.46	73.5	3.73	5.36
41	2.82	73.2	3.52	5.42
37	5.87	71.6	3.56	5.47
42	6.00	72.6	3.50	5.35
43	5.06	71.3	3.51	5.35
44	14.4	65.0	3.17	5.45

Among the yearling steers one observation is worthy of note. Two of these steers were of extra high finish and were graded High Choice. They were numbers 37 and 44 and showed a very white fat similar to pork fat in appearance. The soft and oily nature of the back fats of these animals is reflected in their iodine numbers. By the Hanus method they were 60.0 and 57.6 as compared to the more typical beef fat of animals 42 and 43 which appeared similar in hardness and had an iodine number on their pooled back fats of 46.5. Since all four of these animals were similarly fed, it appears that some fundamental changes may occur in the fat metabolism of some extra heavily finished animals.

In Table 2 is shown a tabulation of data of the Station cattle with respect to breed. The dressed carcasses of the Holsteins graded on the average Utility and the

Herefords graded High Commercial. However, on tenderness at 3 days and 15 days *post mortem* there were no differences between breed on a group basis. In view of the previous paper in this series where a very high correlation was observed between market grade and tenderness, it appears that the uniformity in age, feeding management, etc. of the Station cattle has removed the factor or factors responsible for the positive relation between grade and tenderness. In other words, the factors responsible for the carcass grade differences in these cattle had no relation to tenderness.

TABLE 2

A Comparison of Tenderness Scores of Holstein and Hereford Breeds

Holstein				Hereford			
Animal No.	Carcass grade	Tenderness		Animal No.	Carcass grade	Tenderness	
		3 day level	15 day level			3 day level	15 day level
21	High Utility	7.92	7.92	22	High Com.	2.92	5.58
24	Low Utility	3.25	5.58	23	Ave. Com.	5.58	7.25
25	Ave. Utility	3.75	6.58	26	Ave. Good	3.42	6.58
27	Low Utility	3.66	6.58	28	High Com.	4.42	6.75
30	Ave. Utility	3.25	6.83	29	Ave. Good	2.58	5.67
32	High Utility	3.42	6.67	31	High Com.	4.25	7.08
34	Low Utility	1.50	3.17	33	High Com.	3.42	5.92
35	Low Com.	3.25	7.42	36	Low Com.	2.67	7.58
38	Cutter	2.67	7.42	40	High Com.	3.50	6.42
39	Low Utility	3.25	6.92	41	Low Com.	3.92	8.17
Ave. 3.59 ± 0.11; 6.51 ± 0.11				Ave. 3.67 ± 0.11; 6.70 ± 0.11			
Median 3.25 ± 0.11; 6.75 ± 0.11				Median 3.44 ± 0.11; 6.66 ± 0.11			
Average Grade: Utility				Average Grade: High Commercial			

The data of Table 3 have been arranged in order of optical density at 542 mμ of an aqueous meat extract. This is directly related to percent hemoglobin (particularly muscle hemoglobin) by multiplying by 0.84. Tenderness and alkali insoluble protein at 3 and 15 days *post mortem* and increases in tenderness are shown. In setting up this table six animals have been set apart and not included in the large group for statistical study for following reasons: number 21 has an abnormally low hemoglobin content perhaps indicating some nutritional or pathological involvement and also a high initial tenderness; 37, 42, 43, and 44 are yearlings and highly finished and do not fit in the general pattern of the main body of animals—rather poorly finished 2½ year old steers; also some of these had a high initial tenderness. Number 48 is set aside as it is a 5 year old cow of extraordinarily high content of intramuscular fat. In view of the previously reported *(4)* high correlation of alkali insoluble protein with tenderness it is readily apparent that some fundamental differences in these data of the two studies exist. Already these difficulties have been discussed, but it was considered advisable to report these data to indicate the nature of some of the troubles encountered in problems of this kind. Inasmuch as the reproducibility of these determinations within samples was generally satisfactory it perhaps can be said that little or no decrease in the alkali-insoluble matter has taken place during aging. It has also been found that even these data show some correlation of alkali-insoluble protein with tenderness at the 15 day level; a correlation of coefficient approximately —0.35 with twenty degrees of freedom is obtained using for animals 30 and 33 the 3-day values of the alkali insoluble protein instead of the 15 day values which obviously are out of line. No relation is observed between initial tenderness scores and alkali-insoluble proteins.

As one would expect, there is a relation between initial and final tenderness, but it is less than anticipated, +0.54 on the Station cattle alone. This value of the correlation coefficient is close to the 1% level of 0.56 *(7)* with 18 degrees of freedom.

It should be pointed out that the uniformity of the test cattle apparently was the basis for the observation that no relation existed between intramuscular fat and tenderness. Some correlation of these factors was noted in the previous study *(4)*.

When optical density is treated statistically with

TABLE 3

Tenderness and Alkali-Insoluble Protein at 3 Days and 15 Days Post Mortem and Muscle Hemoglobin as reflected in the Optical Density at 542 mμ

Animal No.	Optical Density at 542 mμ	Tenderness		Increase in Tenderness $T_2 - T_1$	Alkali Insoluble Protein	
		at 15 days T_2	at 3 days T_1		15 days A_2	3 days A_1
26	0.419	6.58	3.42	3.17	0.418	0.505
22	0.436	5.58	2.92	2.66	0.434	0.429
23	0.438	7.25	5.58	1.67	0.320	0.421
34	0.459	3.17	1.50	1.67	0.570	0.568
27	0.469	6.58	3.66	2.92	0.409	0.643
25	0.550	6.58	3.75	2.83	0.727	0.722
38	0.555	7.42	2.67	4.75	0.523	0.824
29	0.560	5.67	2.58	3.08	0.431	0.520
24	0.562	5.58	3.25	2.33	0.337	0.467
39	0.572	6.92	3.25	3.67	0.530	0.572
32	0.600	6.67	3.42	3.25	0.373	0.543
30	0.600	6.83	3.25	3.58	0.615	0.441
28	0.602	6.75	4.42	2.33	0.327	0.463
41	0.666	8.17	3.92	4.25	0.293	0.365
33	0.667	5.92	3.42	2.50	0.847	0.447
31	0.689	7.08	4.25	2.83	0.487	0.452
35	0.699	7.42	3.25	4.17	0.401	0.477
47	0.730	7.92	....	....	0.435	....
46	0.751	7.83	....	....	0.334	....
40	0.761	6.42	3.50	2.92	0.562	0.665
45	0.800	7.25	....	....	0.608	....
36	0.948	7.58	2.67	4.92	0.372	0.449
37	0.610	6.42	5.25	1.17	0.437	0.148
42	0.572	8.66	8.00	0.66	0.416	0.580
43	0.463	7.92	5.00	2.92	0.720	0.453
44	0.743	8.00	5.83	2.17	0.541	0.602
48	0.968	4.75	....	....	0.316	....
21	0.283	7.92	7.92	0.00	0.539	0.647

tenderness at 3 days no correlation is noted. However, in relation to tenderness at 15 days, a correlation coefficient of +0.50 is obtained with optical density. This approaches the 1% level with 20 degrees of freedom for the values at the 1% and 5% levels are 0.53 and 0.42 respectively *(7)*. These findings indicate a possible relation between muscle hemoglobin and changes in tenderness. When a correlation coefficient on these factors is determined a value of 0.55 results and appears very significant because with 17 degrees of freedom the 1% and 5% levels are 0.57 and 0.46 respectively.

At this time there is no indication of the fundamental relationship between muscle hemoglobin and tenderness. However, the amount of muscle hemoglobin is directly related to the muscle plasma and its function. Therefore it appears that muscle plasma is related to tenderness and changes in tenderness. This fact lends confirming evidence to the hypothesis of Deatherage and Harsham *(3)* that muscle plasma is a major factor in the tenderness of beef.

There have been reported in the literature numerous histological studies on muscle and the *post mortem* changes in muscle. These changes appear more pronounced in the muscle plasma than in connective tissue. Consequently it is probable that these initial changes in muscle plasma are responsible for improvement in meat tenderness with aging particularly in the earlier stages.

The apparent relationship between muscle plasma, muscle hemoglobin and tenderness, perhaps indicates that a healthy, strong and well nourished animal is the most important factor in determining good meat from the consumers' point of view. Indeed, in this connection the noteworthy investigations of Bull and Rusk *(2)* and Mitchell and Hamilton *(5)* are brought to mind. The former authors showed that muscular exercise did not toughen meat but appeared to improve tenderness and the latter authors indicated that exercise tended to decrease the content of collagen in muscle, while muscle plasma tended to increase with exercise.

If the results reported here and in the previous study *(4)* are to be used as a basis for further investigation on meat quality as reflected in tenderness, then factors which influence the alkali-insoluble proteins (connective tissue) and the muscle plasma (muscle hemoglobin) must be scrutinized. Quite possibly this may lead to investigations of genetic factors, and feeding management practices, as well as to fundamental studies in biochemistry and physiology. Indeed, an integrated program wherein meat animals are studied from breeding to the table is called for. Such a program can be undertaken only with the earnest cooperation of those interested in all phases of meat, as the livestock breeders, feeders, processors, merchandisers, and consumers and educational and research institutions.

Summary

Twenty-eight cattle have been studied with respect to the biochemistry of tenderness. Included in the group were 10 Hereford and 10 Holstein steers of approximately 2½ years of age and fed in the same manner for one year prior to slaughter; four yearling steers highly finished and full fed since birth; and four market cattle.

Tenderness was determined at 3 and 15 days *post mortem*. In all cases the tenderness increased. There was a significant but not close correlation between initial and final tenderness values.

In the cases of eight animals there were no significant changes in the nitrogen soluble in trichloracetic acid and in zinc hydroxide between 3 and 15 days *post mortem*.

In the twenty identically fed animals there was no correlation between tenderness at 3 or 15 days and carcass grade or intramuscular fat. In two out of four full fed and highly finished yearling steers a peculiarly soft back fat was observed which was reflected in higher iodine numbers.

On the basis of breed, and after identical feeding management, 10 Holsteins and 10 Hereford steers 2½ years old showed no differences in tenderness. There was, however, a difference in carcass grade. Tenderness at 3 days did not show any relation to alkali-insoluble protein. There was some correlation with tenderness at 15 days, but not as high as previously reported.

Muscle hemoglobin showed no relation to tenderness at 3 days but a very significant correlation was observed with tenderness at 15 days and with increases in tenderness between 3 and 15 days. These observations are interpretetd as indicating the importance of muscle plasma in the tenderness of meat.

It appears that both connective tissue as represented by alkali-insoluble protein and muscle plasma as represented by muscle hemoglobin are in part responsible for tenderness in meat.

Acknowledgment

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Factors Affecting the Jelly Grading of Pectin[a]

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The assessment of jelly grade of pectin by means of the Exchange Ridgelimeter has been studied with special reference to the conditions of jelly preparation. The factors investigated have included conditions of cooling, time and temperature of aging, percentage soluble solids content of jelly, buffer salt solution as opposed to natural fruit juice, pH and stage of addition of acid.

The predominating factor appears to be pH, but rate of cooling and temperature of aging may significantly affect the strength of pectin jellies. At pH values above 3.0-3.2, the jellies show increasing sensitivity to changes in aging temperature irrespective of the type and quantity of buffer salts present. Jellies of 70% soluble solids content have been found to be much more stable in this connection than those of 65% soluble solids content.

The laboratory testing of substances in colloidal systems presents difficulties which were particularly emphasized in the papers dealing with pectin grading, given before the Institute of Food Technology in Philadelphia in 1948 *(3, 6, 8, 9)*. It was shown, for instance, that any one sample of pectin might give jellies of varying strength according to the conditions of their preparation. In addition, the various types of apparatus which have been described for the measurement of strength of pectin jellies may estimate different physical characteristics and therefore may give different results on identical jellies. The importance of applying a standard method for the assessment of jelly strength of pectins was consequently recognized, and, with this object in view, the factors influencing the strength of pectin jellies were investigated, and the results are described in the present communication. It was decided to concentrate in the first instance on the effect of variation in conditions of preparation of the jelly, and consequently one type of apparatus only was adopted for measurement of strength. The Exchange Ridgelimeter described by Cox and Higby *(5)* was chosen, mainly because of its simplicity of use, reproducibility of the results, and the fact that, since relatively small quantities of jelly are needed for each test glass, the effect of varying conditions on one single batch can readily be studied. We have also found this apparatus to give results bearing a constant relationship to those obtained when the English B.A.R. instrument *(4)* is used. This relationship may be expressed by the equation

$$y = 49.4 - 20.8 \log x,$$

where y = percent sag Ridgelimeter and x = ml. water B.A.R.

It was obviously necessary in these investigations to start with a basic recipe for the jelly preparation, and it was decided to adopt that which had been used in our laboratories for many years. This recipe had been devised to conform as nearly as possible to average conditions of jam manufacture existing in English factories. In this method the pectin was first dispersed in hot sugar sirup. As Baker and Woodmansee *(2)* have pointed out, it is essential that dispersion should be complete, and we have found that examination under the microscope is the best means of determining whether or not this has been effected. The pH was so adjusted that the final jelly had a pH of 3.00 ± 0.05 and the pectin dispersion then mixed with a solution of sucrose in distilled water and with sodium citrate buffer. The mix was boiled rapidly for 14 ± 1 minutes. Although this time is longer than that normally taken for the boiling of jam in the factory it was originally adopted in order to allow for the slower rate of cooling of jam in bulk in the factory compared with the relatively rapid cooling of the smaller quantities of jelly used in the laboratory. The weight of distilled water taken was such that, in the specified time of boiling, a percentage soluble solids (by refractometer) of 70.0 ± 0.5 was obtained in the final jelly. After pouring into the test glasses, the jellies were aged at 2 to 4° C. for 18 hours and then, immediately after removal from the incubator, tested by the Ridgelimeter.

Calculation of Jelly Grade

It will be realized that calculation of jelly grade according to the American definition (i.e. the number of pounds of sugar supported by one pound of pectin in a standard 65 percent soluble solids jelly) was complicated by the fact that in our experiments the gels were tested at 70 percent soluble solids, in order to conform with the average figure for English jams. To avoid this difficulty, we calibrated the Ridgelimeter against a large number of jellies prepared by our method from many different standardized pectins from reputable American firms. It was found that, on an average, jellies made from pectins which were claimed to be 100 grade gave a sag of 24.4 percent when used in 0.8 percent concentration. Consequently, the jelly grade was defined as 0.8 x 100/percent concentration of pectin in a jelly giving 24.4 percent sag. When studying the effect of varying conditions on jelly grade, it was obviously impossible to ensure that the strength of the jellies would conform to 24.4 percent sag. Use was therefore made of a true/assumed grade curve within what we consider to be the limits of sensitivity of the Ridgelimeter, namely, 22-27 percent sag.

Time of Boiling Jelly Mixture

The factor first considered in the present investigations was the boiling time of the pectin mixture. Several apple and citrus pectins from different sources were made into jellies according to the method described above, except that the time of boiling was varied in different experiments between 3 and 20 minutes. It was found that the majority of pectins were surprisingly stable under the conditions of the test and did not vary

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in strength within a range of approximately 3-16 minutes. After about 20 minutes' boiling, some loss of strength appeared to take place. In view of these results it was subsequently decided to adjust the time to 10 minutes.

Time of Aging Jellies

The effect of aging on the strength of pectin jellies was first studied by Baker *(1)* and Tarr *(14)*, and Baker and Woodmansee later recommended *(2)* an aging period of 24 hours for sag measurements. Other periods recommended have been between 18 and 24 hours *(5, 10, 13)*. In the present investigations pectins from various sources were tested, each sample being treated as follows:

Six glasses were filled from one batch of the pectin jelly and aged at the specified temperature. One glass was removed from the incubator after an interval of 18 hours, and further jellies were tested after 24 hours and then at intervals up to 6 days. This experiment was repeated for different temperatures of aging. In addition to using jellies of 70 percent soluble solids content, tests were also carried out with jellies containing 65 percent soluble solids. The results of the tests are summarized in Table 1. It will be seen that individual rapid set pectins tested between 18 and 24 hours after pouring showed erratic rates of increase in strength, but that after 24 hours of aging this increase became relatively constant and only amounted to 1-2 percent for each subsequent day. Slow set pectins behaved somewhat more erratically, but also showed a more constant rate of increase after 24 hours. It therefore appears that 24 hours is to be recommended as the minimum time of aging which can be regarded as reliable.

TABLE 1

Effect of Time of Aging on Pectin Jellies

Pectin Sample	Percent increase in jelly grade per day from 18-24 hrs. aging		Percent increase in jelly grade per day from 1-6 days aging	
	70 Percent T. S. S.	65 Percent T. S. S.	70 Percent T. S. S.	65 Percent T. S. S.
Rapid set citrus, powder				
D. 20	0.3	5.5	1.6	1.9
D. 75	—2.8	7.3	1.3	1.1
D. 84	2.8	....	1.3	....
D. 89	1.4	3.3	1.3	1.9
D. 99	3.7	....	1.5	....
Rapid set apple, powder				
D. 63	2.9	8.3	1.2	1.6
D. 70	5.8	6.4	1.6	1.5
D. 85	11.7	5.2	1.1	1.4
Rapid set apple, liquid				
FRAC 2	4.4	3.6	1.4	2.3
WRAC 8	0.8	11.5	2.4	2.1
Slow set citrus, powder				
D. 76	6.2	8.2	1.4	3.6
D. 83	9.0	9.8	0.6	3.5
D. 97	4.5	....	1.0	....
D. 98	14.5	4.8	2.8	3.1

*T. S. S. = Total soluble solids.

Rate of Cooling Jellies

At this stage of the investigations, it became obvious that some unknown factor, not covered by the adopted procedure, was interfering, since occasionally results could not be reproduced with rapid or medium set pectins. This was finally traced to the rate of cooling the jellies. If, for instance, after pouring into the testing glass the jelly was rapidly cooled in water to the specified temperature before aging, then the final strength was significantly different from that of a jelly placed in the incubator immediately after pouring, even though both had a total aging time of 24 hours. This difference was particularly great at temperatures of aging around 20-30° C. It was then found that satisfactory duplicates could be obtained if the test glasses, immediately after being filled, were aged for 2 hours at room temperature (i.e. 18-22° C.) before placing in the incubator for a further 22 hours. During the initial 2 hours of cooling it was necessary to keep the glasses separated from one another, so that air could circulate between them. This modification was adopted for all subsequent experiments.

RAPID SET PECTINS

Temperature Effects in Aging and Testing Jellies of 65 Percent and 70 Percent Soluble Solids Contents

The temperature at which jellies should be aged prior to testing does not appear to have received much attention, although Lampitt and Money *(7)* investigated the effect of temperature variation on jellies aged for 4 hours only. Baker and Woodmansee *(2)* recommended 30° C., and "room temperature" has been used by Cox and Higby *(5)*, Mottern and Karr *(10)* and Owens, Porter and Maclay *(13)*. In the basic method adopted for the present investigations, the jellies as stated above were held at 2-4° C. It was therefore decided to see how far temperature of aging affected the jelly grade of pectins, and tests were first carried out on a sample of medium to rapid set apple pectin, using jellies of both 65 percent and 70 percent soluble solids content. A batch was made under the standard conditions which had been adopted, and distributed between six Ridgelimeter glasses. After allowing all the glasses to cool for 2 hours at 18-22° C., one glass was placed in each of 6 incubators held at different temperatures between 2 and 38° C. It was decided to test the jellies at the temperature of aging and consequently, 24 hours after pouring, they were all removed from the incubators and the strengths immediately determined. Typical results are shown in Figure 1. It is seen that, although at 2° C. and 8° C. lower figures are obtained, between 17° C. and 38° C. the temperature at which the jelly is aged and tested has very little effect on the final grade. The jellies containing 65 percent soluble solids are seen

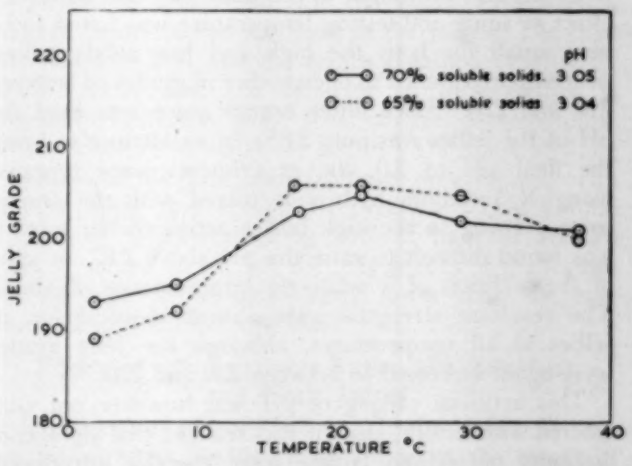

FIG. 1. Effect of Temperature of Aging and Testing on Jelly Grade, Medium and Rapid Set Pectin.

to behave in a similar way to those containing 70 percent soluble solids.

Effects of pH, Synthetic Buffer Salt Solutions, and Fruit Juices

It was realized that this temperature effect might vary according to the type and quantity of buffer salts present, since several workers *(2, 11, 12)* have found that the so-called optimum pH is sensitive to such changes. Using the same pectin as that taken for the tests relating to Figure 1, the sodium citrate buffer was therefore replaced by depectinized Seville bitter orange juice equivalent to 5 percent by weight of the final jelly. This was introduced into the mix at the same point as the buffer salt solution, i.e. before boiling down the jelly. The effect of temperature of aging was again determined as previously, jellies of both 65 percent and 70 percent soluble solids content being tested.

The results are shown in Figure 2. The effects of variations in temperature are negligible between 2° C. and 36° C. for both types of jellies. In addition, the average jelly grade of the jellies of 70 percent soluble solids content is approximately 3 percent higher than those of their counterparts with sodium citrate buffer (compare with Figure 1).

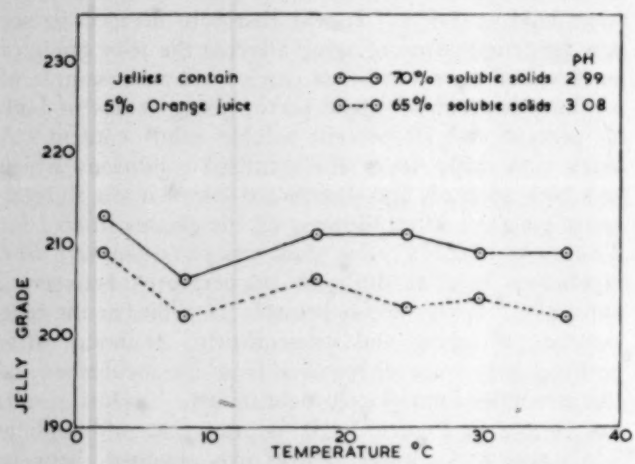

FIG. 2. Effect of Orange Juice on Jelly Grade, Medium and Rapid Set Pectin.

The concentration of orange juice was then increased to 30 percent by weight of the final jelly and again the effect of aging and testing temperature was found to be very small for both the high and low solids jellies, which approximated to one another in grades of between 210 and 220. Since bitter orange juice was used the pH of the jellies was only 2.65. In an attempt to bring the final pH to 3.0, the experiments were repeated using N/1 sodium hydroxide mixed with the orange juice. Owing to the high buffer action of the juice it was found difficult to raise the pH above 2.87, in spite of the addition of a relatively large amount of alkali. The resultant strengths were almost identical for all jellies at all temperatures, although the jelly grades were again increased to between 220 and 230.

This artificial raising of pH was however not considered satisfactory, since it was realized that significant amounts of sodium citrate were thereby introduced. The difficulty of obtaining a high fruit juice content without artificial buffering, whilst maintaining the pH of the jellies at 3.0, was finally overcome by mixing together equal quantities of depectinized bitter orange juice and apple juice, and using the mixture in amounts equal to 30 percent of the total juice in the final jelly. Almost exact duplicates of the results in Figure 2 were obtained for the jellies of both 65 percent and 70 percent soluble solids content.

The effect of high concentration of apple juice alone was then studied, using jellies containing no synthetic buffer salt solution but only apple juice in sufficient quantity to give 30 percent of juice in the final jelly. The pH of the jellies of 70 percent soluble solids content was 3.33, and that of the 65 percent solids jelly 3.34. In these experiments the results recorded differed from those obtained under previous conditions of test (Figure 3). The strengths of the jellies of 70 percent soluble solids content are seen to be lower than

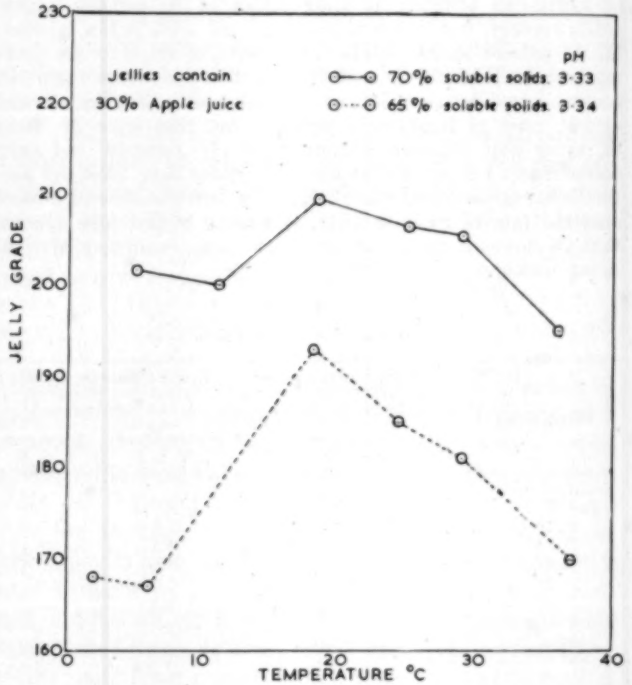

FIG. 3. Effect of Apple Juice on Jelly Grade, Medium and Rapid Set Pectin.

those of their counterparts in the other experiments with fruit juices in this series, while the strengths of the jellies of 65 percent soluble solids are even more strikingly decreased. This is seen to result in a large gap between the curves for the high and low solids gels. In addition, temperature of aging and testing is now found to be an important factor, especially with the lower solids jellies, and a pronounced peak is seen at about 18° C. with minimum strengths at low and high temperatures. That this effect is essentially one of pH rather than of type of buffer salts was demonstrated by repeating these experiments with the same amount of apple juice, but mixing sufficient N/1 hydrochloric acid with the juice to reduce the pH of the jelly to 3.0. The results gave a graph almost identical with that in Figure 2. Jellies made with other fruit juices of relatively high pH, such as apricot, were found to behave

in a manner similar to those prepared with the apple juice.

The relative importance of pH compared with the type of buffer salts was further shown by experiments carried out with the synthetic buffer salt solution suggested by Olsen, Stuewer, Fehlberg, and Beach *(12)*. This solution, when used in the recommended quantities, gave jellies of pH 3.48 with the particular pectin under test. The jelly grades of the 70 percent soluble solids gels were substantially lowered compared with all previous jellies tested at about pH 3.0, and ranged from 170 at 5° C. to 180 at 36° C. with a peak of 190 at 24° C. The effect on the jellies of 65 percent soluble solids content was even more striking, and the jelly grade was reduced to about 110 at high and low temperatures of aging, reaching a maximum of about 140 at 24° C. These experiments were then repeated, adding, however, sufficient acid to the buffer salt solution to lower the pH of the final jelly to 3.3. This resulted in increased strengths of the jellies of both 65 percent and 70 percent soluble solids content (see Figure 4),

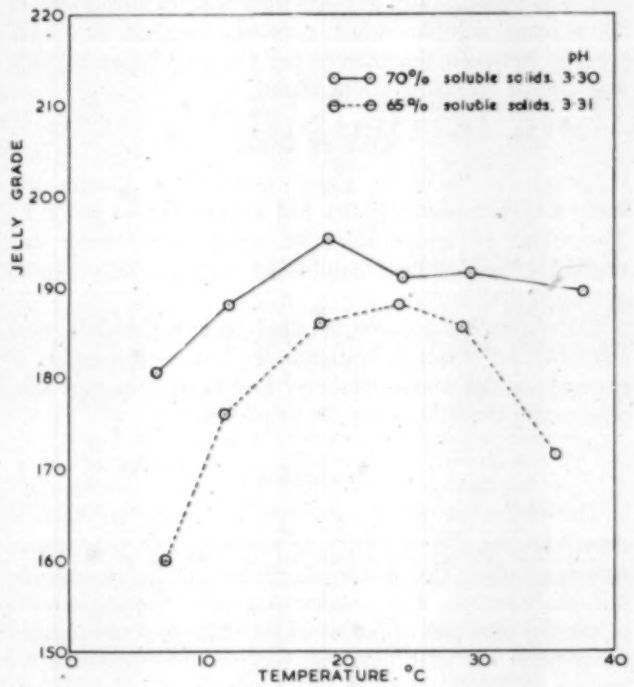

FIG. 4. Effect of Olsen's Buffer on Jelly Grade. pH 3.3, Medium and Rapid Set Pectin.

and decreased the gap between the curves for the two types of jellies, whilst a marked temperature effect was still apparent with the low solids jellies. The tests were then repeated using sufficient acid to reduce the pH of the jellies to between 3.0 and 3.1 (see Figure 5). It will be seen that the jelly grades of all jellies were raised, the gap between the results for high and low solids jellies somewhat diminished, and the temperature peak flattened out.

Acid in Glass,

Although the present investigations are essentially concerned with jellies prepared with acid added to the mix before boiling, it may be of interest to record that the effect of temperature of aging and testing on results

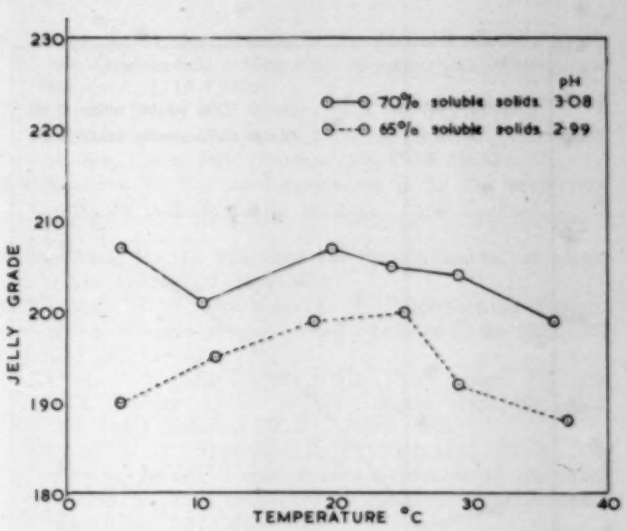

FIG. 5. Effect of Olsen's Buffer on Jelly Grade. pH 3.0-3.1, Medium and Rapid Set Pectin.

obtained with the "acid in glass" method recommended by Joseph and Baier *(6)* was also studied, using jellies of 65 percent soluble solids content. The pH of the resultant jellies was only 2.33. The jelly grades at the different temperatures showed variations between 223 and 227 only, and indicated that temperature at which the jellies are held and tested has a negligible effect in this method.

As previously mentioned, all the above experiments were carried out on one sample of medium to rapid set apple pectin. The general findings have, however, been confirmed, not only with other samples of rapid and medium set apple, but also with medium and rapid set citrus pectins. The only difference noted was that jellies of 65 percent soluble solids content made from citrus pectin appear to be more sensitive to temperature of aging than are similar jellies made from apple pectin.

SLOW SET PECTINS

Temperature Effects in Aging and Testing Jellies of 65 Percent and 70 Percent Soluble Solids Contents

Samples of slow set powdered citrus pectin were then tested, in order to determine whether or not these behaved similarly to rapid set pectins at different temperatures of aging, and, for the initial experiments, sodium citrate buffer was used in the jellies. The final pH was 3.09-3.18, and the results obtained (Figure 6) show clearly that the behavior of these jellies is markedly different from that of the corresponding jellies made from rapid set pectin. The most striking effect is that the jelly grade increases progressively with rise of aging and testing temperature. This finding applies to jellies containing both 65 percent and 70 percent soluble solids, but the jelly grades of the former are all seen to be significantly lower than the corresponding grades of the latter.

Effects of pH, Synthetic Buffer Salt Solutions, and Fruit Juices

Replacement of the sodium citrate buffer by depectinized bitter Seville orange juice, to the extent of 5 percent of the final jelly, had a negligible effect both

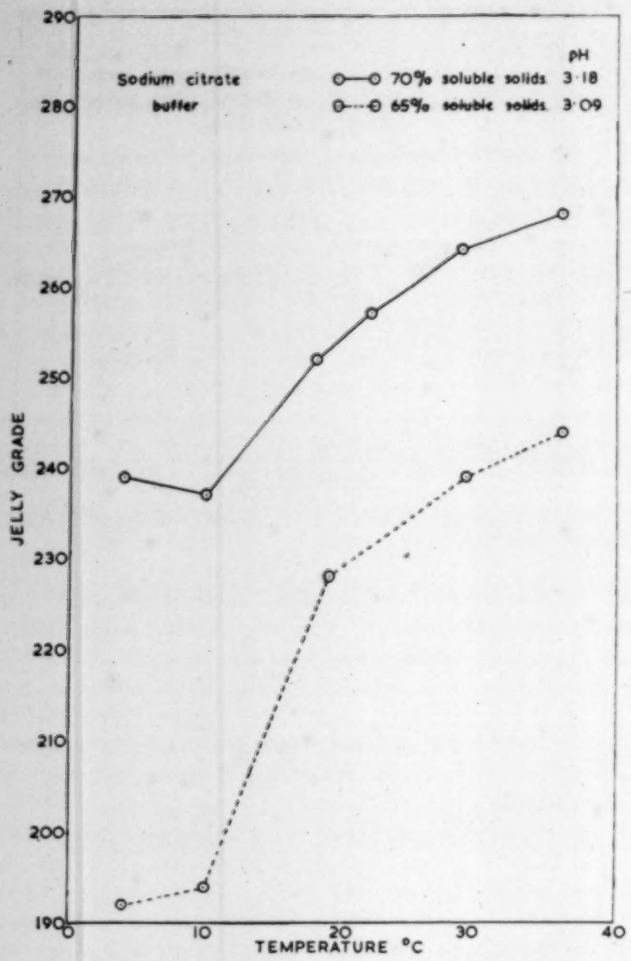

FIG. 6. Effect of Temperature of Aging and Testing on Jelly Grade, Slow Set Pectin.

on pH and on jelly grade at all temperatures. When however the amount of orange juice was increased to 30 percent of the final jelly, the pH fell to about 2.8 and the shape of the graphs became significantly changed. The effect of temperature was eliminated and the jelly grades of the pectin in all jellies of the same soluble solids content approximated to one another at about 293. The jelly grades in the jellies containing 65 percent soluble solids were affected to an even more striking degree, and an almost straight line graph, parallel to the abscissa, was obtained at the same level as the higher solids jellies, namely, at about 293 jelly grade. It was realized that this phenomenon might have been the result either of change in quantity or composition of the buffer salts or of change in pH. To investigate this point further, the experiments were repeated with sodium citrate buffer, using however sufficient N/1 hydrochloric acid mixed with the buffer to give jellies of the same pH as those prepared with 30 percent orange juice i.e., 2.8.

Exactly the same type of graph was obtained as with the 30 percent orange juice series, the only difference being that the jelly grades were all lowered, being 265 for the jellies with 65 percent and 275 for the jellies with 70 percent soluble solids contents.

The relative importance of pH, compared with type and quantity of buffer salts, in stabilization of jellies towards temperature of aging was confirmed by experiments using depectinized apple juice. The juice was added in quantities equivalent to 30 percent of the final jelly, which had a pH of 3.4. A marked temperature effect, comparable to that obtained with sodium citrate buffer at pH 3.1-3.2, was found with jellies of both 65 percent and 70 percent soluble solids contents. The jelly grades were however all considerably lower than those of the corresponding sodium citrate jellies at the higher pH, being 20 to 30 points lower in the jellies containing 70 percent soluble solids and 80 to 100 points lower in the case of the jellies containing 65 percent soluble solids. When sufficient acid was mixed with the apple juice to lower the pH of the jellies to about 2.8, the effect of aging temperature again became eliminated. The jelly grades at all temperatures approximated to one another at a level substantially higher than found at the higher pH. The increase in strength of the jellies of 65 percent soluble solids content was significantly greater than that of the jellies of 70 percent soluble solids content, so that the wide spacing between the curves for the two types of jelly was almost completely eliminated.

Acid in Glass

Using the "acid in glass" method of Joseph and Baier *(6)*, the pectin under test gave jellies of pH 2.37. The effect of temperature of aging and testing was negligible under these conditions and the jelly grades averaged 280.

Unfortunately no slow set apple pectin powders were available for these investigations, but confirmation in general of the above findings has been obtained with other samples of slow set citrus pectin.

Discussion

The object of the present investigations has been to determine the effect of varying conditions of jelly preparation on the jelly grade of pectins. The results obtained, however, demonstrate not only the importance of careful selection of points of procedure in any method formulated for jelly grading, but also the sensitive behavior of jellies under some conditions compared with their relative stability under others. The dominant factor in this connection appears to be pH, and, at a pH in the region around 3.0, neither temperature of aging and testing in the range studied (2-38° C.) nor soluble solids content in the range studied (65-70 percent) has been found to affect significantly the jelly grade of the pectin under test. Above pH 3.0, however, jelly formation appears to be more susceptible to changes in aging temperature. This is particularly noticeable in the case of jellies containing 65 percent soluble solids. The greater stability of jellies of 70 percent as compared with 65 percent soluble solids contents is considered to be a point of practical importance in addition to being of academic interest.

Aspects of jelly preparation which have not previously received detailed attention are rate of cooling and temperature of subsequent aging and testing, and

it would now appear that these factors should be standardized in methods for estimating the jelly grade of pectins.

Acknowledgments

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Industrial and Legal Viewpoints

Chemical Agents and Residues in Foods: From the Viewpoint of the Law[a]

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The general interest in the legal control of the use of chemical agents in the production of foods and as functional ingredients, is highlighted by current consideration of this question in legislative, administrative, and industrial circles. These usually imply consideration of Federal laws and primarily the Federal Food, Drug, and Cosmetic Act. The relevant provisions of these laws are discussed. They should be adequate to deal with the problem, but for the fact of impracticability of enforcement. While some amendments to the law may, therefore, be indicated, the problem is not as acute as alarmists would have us believe. The record of the food industry and food technologists in the use of chemicals in foods and food processing is one of which the industry may be justly proud.

The general interest in the use of chemical agents in the production of foods and as functional ingredients in food processing is highlighted by two recent developments. One of these is the meeting held last December under the auspices of the Food Nutrition Board of the National Research Council as reported in the last issue of your official journal. The chairman of this conference was requested to appoint a committee at an early date to complete organizational and operational plans looking towards the creation of a body which would provide the scientific guidance with respect to the use of the newer chemicals in foods. The other development to which I have reference is the approval by the Rules Committee of the House of Representatives, on May 11, 1950, of a resolution calling for the investigation of the adulteration of food products by the use of new chemicals. The former concerns itself with the problem of adequate pretesting of new chemicals before they are offered to the general public in the form of new food ingredients, and the latter with the statutory need to require such pretesting. In view of the keen and general interest in this problem as evidenced by these two developments as well as others, the inclusion of the subject of existing legal requirements with respect to chemical agents and residues in foods as a part of your consideration of the general question, is most timely.

Of significance is the fact that in all of these discussions of this so-called "problem," the term "chemical" is not defined more precisely than by the term "newer chemicals." In its broader sense, the term "chemical" includes every food and every food constituent. Obviously, we are not concerned with chemicals as identified in this broader sense when we concern ourselves with technical problems of their suitability and safety as food ingredients. Its scope is immediately restricted when we limit ourselves to these considerations, because then

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we are primarily concerned with those chemicals whose relationship to food is non-existent or so remote that their suitability and safety cannot be evaluated on the basis of past experience. It is that kind of substance which is primarily intended when the provisions of the law are considered which regulate the use of chemicals in foods.

Whenever we have occasion to consider the legal restrictions upon, and requirements for, using chemicals in foods, we think almost altogether of the Federal laws. It is true that each State has its own health laws which regulate the use of chemicals in foods in some way. However, I can state from personal experience that, subject to a few exceptions based on a special treatment of certain substances for particular uses, the State officials follow Federal recommendations and the tendency to do so increases. Therefore, we are primarily concerned with the provisions of the relevant Federal laws. Those of these laws which have application to our question are the Federal Food, Drug, and Cosmetic Act, the Meat Inspection Act, and the Federal Insecticide, Fungicide and Rodenticide Act (sometimes referred to as the "Economic Poison Act").

In considering regulatory problems pertaining to the use of chemicals in foods, we are, of course, primarily concerned with their effect upon or in a food as revealed when that food reaches the dinner table. Since the Federal Food, Drug and Cosmetic Act is the law which is designed to insure safe and honest foods on the dinner table, this Act is the one with which we should be particularly acquainted, since we have little, if any, interest in using a chemical in a food if such use will serve to close the channels of interstate commerce to such food by reason of some provision of this Act. The Meat Inspection Act is of like importance when chemicals are considered for use in meat or meat products covered by it. The Economic Poisons Act should also be considered because the residues of the substances regulated by it are subject to the provisions of the Food and Drug Act. I will, therefore, also consider these two Acts briefly.

The provisions of the Meat Inspection Act parallel those of the Food, Drug, and Cosmetic Act in their application to meat and meat products produced under the former. Indeed, these two laws overlap. The extent to which they do overlap has not been judicially determined and is not likely to be, as long as the present interagency cooperation continues to function as it has and does. The Meat Inspection Act includes a provision authorizing the Meat Inspection Division to exclude chemicals from meat and meat products produced under this Act, which will render such meat and meat products "unsound, unhealthful, unwholesome, or unfit for human food." In making their administrative determinations of compliance with these provisions, this agency works very closely with the Food and Drug Administration officials, and, for all practical purposes, the requirements by the Food and Drug Administration for justifying the use of chemicals in foods under their jurisdiction, are the requirements which will have to be met when a chemical is proposed for use in meat and meat products.

The Meat Inspection Division also has substantially the same requirements as the Food and Drug Administration for inclusion of chemicals on the basis of their usefulness in meat and meat products. The statutory authorization for administrative decision as to when a substance, recognized as safe, should or should not be included in meat, is quite obscure. The whole pattern of regulations governing the use of such substances in meats stems from the labeling provision of the law and particularly from those which require that these products be not sold "under any false or deceptive name." The question of authority to prohibit or restrict the use of safe substances in such meat products by reason of this statutory provision was first raised in the now famous "Sausage Case." The Secretary of Agriculture had undertaken to restrict the quantity of moisture and cereal which might be used in any product sold as "sausage." His authority to do this was challenged. The case went to the Supreme Court where it was held that a regulation fixing the maximum percentage of these constituents of sausage bore a reasonable relation to the statutory requirement that a meat product be sold under a name which was not deceptive; and that the question of fact whether the use of the name "sausage" on a product containing water and cereal in excess of the specified quantity would be deceptive, was one for determination by the Secretary. This, then, is the legal basis for the Meat Inspection Division's requirement that no ingredient, including one admittedly safe, may be used in any meat or meat product produced under the jurisdiction of the Meat Inspection Division, unless prior approval of such use has been given.

The Economic Poisons Act is not primarily a consumer statute. It is calculated to insure the efficacy of substances in killing insects, plants and rodents which are not wanted; to safeguard those which are wanted; and to protect the users of these substances against possible injury. However, to the extent to which residues of any of the substances regulated by this Act remain in, or otherwise affect, any food as offered for human consumption, the use of these substances become subject to the regulatory provisions of the Federal Food, Drug, and Cosmetic Act. Therefore, when the food technologist considers the use of an insecticide or a fungicide for the purpose of bringing a food to maturity, he should, at the same time, determine whether a residue of that substance will remain in such food and how such use and the residue, if any, reveal themselves in the food as offered for human consumption. The basic legal requirements of the Federal Food, Drug, and Cosmetic Act, should, therefore, be also considered when a chemical is proposed for the primary use of aiding in bringing a food to maturity.

We come, then, to the consideration of the relevant provisions of the Federal Food, Drug, and Cosmetic Act. For the purpose of this discussion we may divide the chemicals under consideration into two groups; (1) those which are recognized as safe for use, but the presence of which may serve to adulterate the food; and (2) those which might well serve a useful function in a food, but the safety of which for such use is questionable or unknown.

There are, no doubt, many new chemicals available for use as food ingredients over which the sales manager and the copywriter wax enthusiastic. However, sometimes the very enthusiasm from these sources should serve as a warning to the food technologists that the precise effects on the food for which the product is intended, be carefully scrutinized and the results considered with their lawyers. For example, I have in mind the use of a chemical in flour intended for use in yellow cakes and egg noodles. The effect of this chemical is such as to produce a golden yellow color in finished baked products, a color otherwise obtainable, and obtained by housewives in home cooking, only by the use of a substantial quantity of egg. The Food and Drug Administration has put an end to the use of this chemical in flour. Such use constitutes a violation of the law because the end product is economically adulterated in that it is made to appear better and of greater value than it is. Under the law no substance may be used in a food which will serve to constitute that food a dishonest food, or, in the language of the statute—

> "If damage or inferiority has been concealed in any manner; or if any substance has been added thereto or mixed or packed therewith so as to increase its bulk or weight, or reduce its quality or strength, or make it appear better or of greater value than it is."

I have heretofore stated on another platform that any substance which is innocuous and which does not run afoul of these statutory provisions, many be used in a food. Officials of the Food and Drug Administration are a little more conservative, I am afraid. They insist, as many of you have, no doubt, found out, that use of such a chemical must be justified by an affirmative showing that such use is of benefit to consumers. That sounds rather harmless on its face and is quite acceptable as an abstract proposition. No one wants to incur added expense simply for the sake of doing so; hence no substance is ever proposed for use unless it serves a useful purpose in the opinion of the person spending the money for it. However, there can be very serious disagreement as to whether certain effects are affirmatively beneficial or not. It is my view that this should be left for determination by the user, once it has been established that the substance is innocuous and that it will not serve to affect the integrity of the food adversely. But, as stated, the regulatory officials usually insist on a little more than this.

In considering the suitability of a chemical in a food from the standpoint of safety, the legal requirements are of a more technical character and are not always clearly understood by those who propose them for use as food ingredients.

The law closes the channels of interstate commerce to a food "if it bears or contains any poisonous or deleterious substance," subject to certain conditions and exceptions. In considering the safety of a chemical, it must, therefore, first be determined whether it is "poisonous or deleterious" within the meaning of this statutory language. If it is not, its use need only be squared with the economic adulteration provisions considered above. If it is, then certain statutory restrictions and conditions must be met.

Since almost any substance is capable of producing some harmful effect under given circumstances, it is most difficult to define the words "poisonous or deleterious." They are not defined in the statute nor have the regulatory officials issued an interpretative definition which would serve as a guide to those who have to come to a conclusion as to their applicability to a given substance under consideration for food uses. However, it is generally understood by those having had occasion to resolve this question in specific cases, that the Administration bases its decisions as to whether a substance is poisonous or not, largely on the layman's understanding of this term; that is, whether the substance, when consumed as such, in a quantity reasonably capable of ingestion, will cause harm. I might be a little more specific than that by saying that the LD_{50} of sodium benzoate is somewhere close to the dividing line between substances which are "poisonous" within the meaning of these statutory provisions and those which are not. However, such a line of demarcation has never been specifically recognized, and it is readily apparent that it cannot be generally recognized because, while the LD_{50} and acute toxicity data have value in making certain determinations, they are not accepted as adequate data to draw conclusions as to chronic toxicity. The only safe procedure is clearance with the regulatory officials on the basis of the specific facts of each case.

Substances which are thus considered "poisonous or deleterious" may be classified into four categories by virtue of the relevant statutory provisions, namely—

(1) Added substances which *may* render a food injurious to health.

(2) Substances naturally present in a food in a quantity so as to *ordinarily* render such food dangerous to health;

(3) Added substances which are not "required" in the production of a food; and

(4) Added substances which are "required" or cannot be avoided by good manufacturing practices, and are restricted to a quantity not likely to render such food injurious to health, or, if a legal limit has been fixed by regulation, not in excess of the prescribed quantity.

The first category requires no discussion. Obviously no one will want to use any chemical which may be injurious to health. There can be violent disagreement, however, as to when such is the fact, but that does not present a legal but a factual question for resolution by toxicologists.

The second category contemplates such substances as, for example, hydrocyanic acid found in Burma beans, and some of the toxic substances found in mussels and clams during certain seasons of the year. Caffeine is a substance falling in this category. It may be considered "deleterious" for the purpose of this provision of the law, but it is not present in sufficient quantity in the foods in which it is found so as to render those foods "ordinarily" dangerous to health.

The third category confronts us with another undefined term. We must decide whether the substance